Testing the potential of priority effects to sustainably increase community function in semi-natural grasslands

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Short Summary

A long lasting yet still ongoing debate in community ecology is the question on what are the prevalent forces which drive community assembly. Although ecologists agree that environmental factors are of importance in structuring (plant-) communities many studies found evidence for stochastic events (e.g. disturbance and the subsequent recolonization of a habitat) being the major driver during community assembly. One specific aspect of stochastic events which particularly involve the sequence in which species arrive and (in further) interact at a certain location are called priority effects.

The topic of this dissertation is the investigation of priority effects in plant communities, more detailed, the potential for priority effects to influence central European semi-natural grasslands in terms of functionality (biomass production), species composition and the interplay of both.

This has implications for the general understanding of (plant-) community assembly, restoration practise, and land management as well as for conservation of central Europe's semi-natural grasslands. This dissertation project aims at using knowledge from ecological research which has been gathered over the last decades and its application of recent concepts originating from community ecology, biodiversity research, plant ecology and agricultural practise.

For this purpose, several experimental approaches were applied (ranging from greenhouse- to field experiments, see chapter "A ladder of experiments") which aim to elucidate the effects and the underlying mechanisms through which priority effects may influence model plant communities (both, artificial and semi-natural grasslands). At the same time, each one of these experiments involved at least another factor added to the design to further elucidate the quality of mechanisms responsible for the impact of priority effects on (model-) ecosystem function.

Within the four papers included in this dissertation priority effects are induced by either the sowing of different starting communities (and community size) or by an experimental variation of arrival times of different plant functional types (PFTs: legumes, forbs, grasses). Thereby I point out, that an increase in

community productivity can be achieved as a result of priority effects by sowing legumes prior to other functional groups. And, that this effect is stronger than effects of increased sowing density or time interval between sowing events. After these insights were obtained from greenhouse experiments with model communities, priority effects induced by a variation in arrival time of PFTs were tested under field conditions, in regard to community assembly. I could show that the increase in aboveground productivity (when legumes were sown first) was a result of different community biomass allocation patterns between above- and belowground plant parts as a result of the varying order of arrival of PFTs. Thus, there was interplay of belowground asymmetric competition leading to aboveground asymmetric competition in the course of initial community assembly. Additionally, this effect was independent from differences in soil conditions hinting at a higher importance of stochastic events for community assembly.

It can be concluded that the increased productivity of plots with legumes sown first (for the greenhouse experiment as well as for the field experiment) was rather due to the observed differences in biomass allocation than due to direct or indirect nitrogen facilitation because effects of nitrogen facilitation on productivity would have been stronger if sown density of legumes increased. Since this was not the case in the greenhouse experiment mentioned before (where sown density was a factor) nitrogen facilitation seems to not play an essential role during initial community assembly. Additionally, there was no evidence for any positive effect of legume presence on N concentrations (neither in plant material nor in soil samples) or growth rates of legume neighbours in another experimental setup directly aimed at this question.

The results of this thesis enable us to see community assembly not only as the process (or a sum of many different processes) resulting in the patterns we observe when looking at the structure of plant communities. If well understood, priority effects can be used to steer and direct community development into desired trajectories. This could be shown within the methodical boundaries of this thesis, however further research in this direction is necessary to investigate the plasticity of priority effects under different environmental conditions, ecosystems and/or between different organisms.

Kurze Zusammenfassung

Innerhalb der auf Lebensgemeinschaften abzielenden ökologischen Forschung, wird seit jeher darüber debattiert, was die maßgeblich für die Etablierung von Artengemeinschaften verantwortlichen, externen Treiber sind. Obwohl sich viele Ökologen darüber einig sind, dass abiotische Umweltfaktoren eine entscheidende Rolle bei der Zusammensetzung von (Pflanzen-) Gesellschaften spielen, deuten einige Studien darauf hin, dass zufällige Ereignisse (z.B. Störung und die darauf folgende Neubesiedlung eines Standortes) wichtiger sind.

Einen besonderen Aspekt der ökologischen Reaktion auf diese zufälligen Ereignisse stellen sogenannte *Priority Effects* dar. Dabei handelt es sich im engeren Sinne um Effekte, die in Abhängigkeit von der Reihenfolge der an einem Standort eintreffenden Pflanzenarten zwischen diesen stattfinden und dadurch die Ausprägung der sich entwickelnden Pflanzengemeinschaften stark beeinflussen können. Die vorliegende Arbeit beschäftigt sich mit der Erforschung von *Priority Effects* in Pflanzengemeinschaften bzw. geht der Frage nach, inwieweit *Priority Effects* die Funktionalität (Produktivität) und Artzusammensetzung von Grünland-Gemeinschaften, sowie das Zusammenspiel von beiden bestimmen.

Diese Dissertation zielt darauf ab, das innerhalb der letzten Jahrzehnte gesammelte Wissen (und seine Anwendung) aus den Bereichen der Ökologie von Lebensgemeinschaften, Biodiversitätsforschung, genereller Pflanzenökologie sowie landwirtschaftlicher Praxis zu nutzen, um die oben genannte Thematik zu erforschen. Die Ergebnisse aus dieser Arbeit haben Relevanz für das generelle Verständnis über die Bildung von Artengemeinschaften und können einen wertvollen Beitrag in Bereichen der Renaturierungspraxis und des Landmanagements, sowie für den Naturschutz in Mitteleuropäischem Grünland liefern.

Zu diesem Zweck wurden mehrere experimentelle Ansätze entwickelt (von Gewächshausversuchen bis hin zu Freilandexperimenten, siehe Abschnitt “A ladder of experiments”) um *Priority Effects* und die zu Grunde liegenden Mechanismen zu erforschen. Jedes dieser Experimente behandelt *Priority Effects* unter Hinzunahme mindestens eines zusätzlichen Faktors um Wechselwirkungen mit einzuschließen und somit

genauere Aussagen über die Plastizität und Auswirkungen von *Priority Effects* auf die genannten Modellsysteme und deren Funktion treffen zu können.

Innerhalb der vier, hier vorgestellten, wissenschaftlichen Artikel werden *Priority Effects* entweder durch die Aussaat unterschiedlicher Ausgangsgemeinschaften (mit unterschiedlicher Artenzahl) oder durch eine Variation innerhalb der Aussaatreihenfolge von funktionellen Gruppen (Leguminosen, (nicht-Leguminosen-)Kräuter, Gräser) erzeugt. Dabei wurde deutlich, dass durch die vorzeitige Aussaat von Leguminosen eine Erhöhung der Gesamtproduktivität von experimentellen Graslandgesellschaften (unter Gewächshausbedingungen) erreicht werden konnte. Zusätzlich konnte gezeigt werden, dass die Aussaatreihenfolge einen deutlich größeren Einfluss auf die Produktivität hatte, als beispielsweise die Menge der gesäten Samen oder die Wahl des Zeitintervalls zwischen den aufeinanderfolgenden Aussaaten.

Nach den Erkenntnissen aus den ersten Gewächshausversuchen wurde dieser Ansatz (der wechselnden Aussaatreihenfolge funktioneller Gruppen) weiter unter Freilandbedingungen erprobt. Diesmal unter Einbeziehung der natürlichen Dynamik zu- & abwandernder Arten. Hier konnte gezeigt werden, dass die Erhöhung der (oberirdischen) Produktivität (wenn Leguminosen zuerst gesät wurden) ein Resultat von unterschiedlichen Allokationsmustern, in Abhängigkeit der Abundanz, der verschiedenen funktionellen Gruppen war. Daraus folgerte ein Zusammenspiel unterschiedlich gewichteter, unterirdischer Konkurrenz, welche sich anschließend auch auf das oberirdische Konkurrenzverhalten auswirkte und dadurch die Artzusammensetzung und Abundanz von Arten (bzw. funktioneller Gruppen) innerhalb der Gemeinschaft beeinflusste. Diese Muster konnte unabhängig von Bodenbeschaffenheiten nachgewiesen werden und betonen die Wichtigkeit solcher zufälliger Ereignisse bei der Entstehung von Pflanzengesellschaften.

Schlussendlich kann davon ausgegangen werden, dass die beobachtete Produktivitätssteigerung in Gemeinschaften in denen Leguminosen zuerst gesät wurden, eher auf die unterschiedlichen Allokationsstrategien der funktionellen Gruppen zurückzuführen ist, als auf einen Stickstoffdüngungs-Effekt der Leguminosen,der mit der Aussaatdichte zugenommen haben müsste. Da dies weder im

Gewächshausexperiment nachgewiesen werden konnte (wo Aussaatdichte eine der Behandlungen innerhalb des Experiments darstellte) noch in anderen Experimenten, die speziell auf diesen Aspekt abgezielt hatten, scheint Stickstoffdüngung durch Leguminosen kein relevanter Faktor in diesem Zusammenhang zu sein.

Die Ergebnisse dieser Arbeit helfen uns dabei die Zusammensetzung von Lebensgemeinschaften nicht nur als bloße Aneinanderreihung von Prozessen zu verstehen, die wir sehen, wenn wir die Struktur von Lebensgemeinschaften betrachten. Bei richtigem Verständnis können *Priority Effects* dazu genutzt werden um in die Entwicklung von Lebensgemeinschaften einzugreifen und diese in bevorzugte Bahnen zu lenken. Dies konnte innerhalb des (methodischen-) Rahmens dieser Dissertation belegt werden aber dennoch bedarf es der weiteren Erforschung von *Priority Effects*, z.B. in unterschiedlichen Klimabedingungen, Ökosystemen und zwischen verschiedenen Organismen.

1. Introduction

The following introductory paragraphs condense (1) the theoretical background of assembly theory and the role of priority effects in community assembly. Additionally I shortly summarize further aspects, relevant to this work such as filter theory, biodiversity effects and links to ecosystem function in grasslands and how these interact with priority effects as well as the relevance of this topic towards restoration practise. After that, I briefly discuss (2) priority experiments in ecology leading over to the current state of art in ecological experiments on priority effects, including an overview of my own experimental activities within this dissertation project, finishing with a timetable for the development and measurements made in the Priority Effect field experiment. Hereafter a (3) summary of the manuscripts of this cumulative thesis is given and how they relate to each other. At the end I mark potential research challenges arising from these manuscripts and provide an outlook on future research directions. The introduction ends with information on the publishing status of each manuscript, including a description of my own contribution. In addition, tables which provide information on presentations of my research at conferences and meetings as well as a table on trainings and workshops I have attended are shown.

1.1 Background

1.1.1 Assembly theory and the importance of priority effects

Per definition, assembly theory is seen as the explicit constraints that limit how assemblages are selected from a larger species pool (Weiher, Clarke & Keddy 1998). The original, yet ongoing debate leading to this current "state of the art" is how exactly communities assemble and what factors, mechanisms and rules apply during the process of species sorting resulting in the patterns we observe in nature. Through the myriads of possible interactions among abiotic factors and species often varying across space and time, this remains a task which aims at a considerably high goal. Nevertheless, disentangling what is decisive for community composition and whether the co-occurrence of species is depending on their

interaction within or between species, in exchange with environmental factors and/or is solely determined by chance events is a key question in community ecology (Connor & Simberloff 1979; Drake 1990).

Jared Diamond (1975) proposed, after observing avian assemblages on islands near New Guinea, that initial community composition (of primarily colonized habitats after disturbance e.g. primary succession) was characterized by a set of "Assembly Rules" which could be predicted from the size of the species pool, the abiotic environment and the interactions between component species. However, Diamond also found, that in some cases community composition varied although these key factors appeared to be similar. By then he suggested that the sequence and timing of colonisation/invasion could have substantial relevance for community assembly and potentially lead to multiple stable equilibria, with some bird species managing to outcompete others and thus limit the realised niche of the other species.

Even long before Diamond there have been contrasting concepts about succession and the generic mode of community development mainly formulated and discussed by the two contemporaries Clements and Gleason (Gleason 1926; Weaver & Clements 1938). In general the dynamics of community assembly provide a solid framework for a general theory of community organisation (Drake 1990). Since our understanding of the patterns of community assembly depend on which ecological theory works as the general framework and whether communities exist in form of single or multiple stable equilibria, the two opposing concepts of Gleason and Clements (as well as the current state of the discussion) will be presented in the following.

Clements (1916) pictured the development of communities to be deterministic and considered them to develop up to a single stable climax stadium which would be primarily a result of the available species pool determined by the present environmental conditions. Based on the theories of Clements, Gleason (1926) formulated an opposing concept in which he highlighted the importance of colonisation and stochastic/random events. Thereby he included the fluctuation of available niches as disturbance occurs within ongoing community development. The importance of historical contingency, in the sense of "which

species' do establish and propagate constitute a community" formed a non-deterministic counterpart to the model of Clements.

Later, Lewontin (1969) postulated a theory which aimed to explain the observed structure of communities. His approach was mainly driven by one of two paradigms: (1) (…) "History is relevant to the present state of populations, species and communities, and that their present state cannot be adequately explained without reference to specific historical events." (2)"The latter approach has as a basic assumption that only one stable point exists", and that communities are steering towards that single climax solely driven by fixed (environmental) forces. These ideas still resemble the main debate about assembly, but recent studies suggest that there are both stochastic and deterministic forces at work. The current challenge is to find out which aspect is the key driver of community assembly at any given time. A task of similar importance is to find out what the effect sizes of random vs. deterministic forces may be along temporal, spatial or biogeochemical gradients.

Sutherland (1974) picked up the work of Lewontin and tested his hypothesis on a range of different community types at Beaufort, North Carolina. He found empirical evidence that the order of arrival of species was a major determinant of later community structure. Thus, he introduced a new, intermediate hypothesis on the nature of community development: The Alternative Stable States Model. In this model communities are restricted and structured in their development to some extent by environmental factors but also contain elements of randomness. Historical factors such as the order of arrival or the sequence of colonization can cause *priority effects*, whereby those species' that arrive first can significantly affect further community structure. If priority effects occur at local alpha diversity scales, this can lead to alternative stable states at slightly larger scales (beta diversity; see Martin & Wilsey 2012a).

Priority effects during community assembly provide the right conceptual framework within which to ask how strong the role of random- versus deterministic drivers of change are in communities. Since then many studies have found evidence for the ability of stochastic events to affect the outcome of community assembly thus resulting in communities composed by different species (Drake 1991; Ehmann &

MacMahon 1996; Palmer, Young & Stanton 2002; Palmer *et al.* 2002; Ejrnaes, Bruun & Graae 2006a; Körner *et al.* 2007a; Jiang & Patel 2008; Fukami & Nakajima 2011b; Kreyling, Jentsch & Beierkuhnlein 2011).

As reviewed by Chase (2003b), there is evidence for both sides. If certain conditions are met, communities tend to be able to develop into alternative stable states (ASS) mainly driven by stochastic processes such as the assembly history of the species contained (Neutral Theory). On the other side there is evidence, that if relevant factors are constrained, communities tend to develop into just one stable state (mainly driven by environmental factors) irrespective of their assembly history and the order of species' arrival. In how far this implies generality and is applicable throughout the vast range of ecosystems, community types, trophic guilds and different environmental constraints existing on earth remains one of the challenges of ecologists (Sutherland *et al.* 2013). However, initial community structure is contingent on the relative arrival times of species (Young *et al.* 2015). Chase (2003) created a framework for synthesis to elucidate under which conditions communities tend to develop into which directions and how this affects alpha-, beta- and gamma diversity (see Fig. 1).

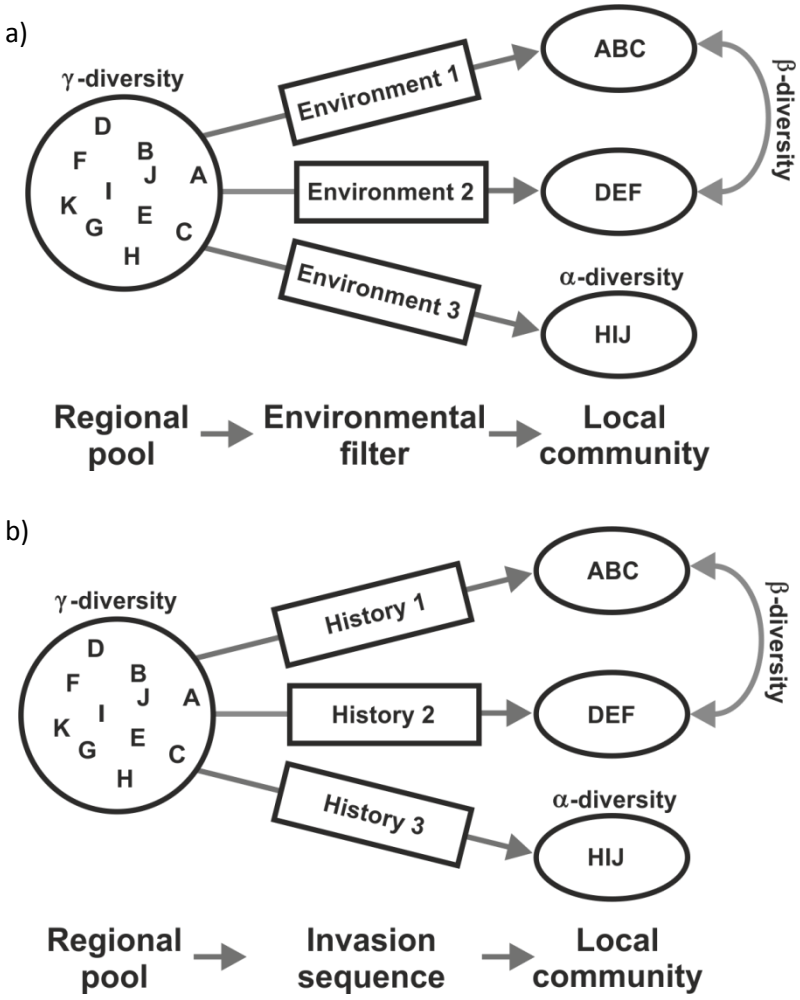

Fig. 1: a) Schematic representation of community assembly when there is a single stable equilibrium for each of several different environments. Differences in species composition from site to site (β-diversity) are the result of different environmental filters. b) Schematic representation of community assembly when there are multiple stable equilibria from different assembly histories (environmental conditions are identical). Local diversity (α-diversity) and composition is the result of the regional species pool and the order in which species enter a community. Differences in species composition from site to site (β-diversity) are the result of different invasion sequences (modified from Chase (2003b)).

However, as Beisner & Haydone (2003) generally follow the same line of thought but point out that there are two different ways to look at ASS. Either they are caused by a shift in variables contained in a system (such as species occurrences, -richness or –abundances) or by a change to parameters (ecosystem perspective) governing interactions of any kind (between species and/or environment). The latter would be caused by changes in environmental conditions (e.g. climate change) and as a result not all kinds of ASS would be realized at all times. In contrast, variables could be subject to change by (local-) perturbations

and thus evolve from one state to another while the original state may still persist where perturbations were lacking. In this case there are two classes of ASS possibly arising from this point: (1) "If the system of equations describing the transformation of a state is nonlinear...there may be multiple stable points with all species present so that local stability does not imply global stability" (Lewontin 1969). (2) However, there may be the case that only a subset of species continues to persist (or species new to the system are being introduced) while others fall victim to (or being introduced by) species interactions (becoming relevant only in course of dynamics altered by the perturbations).

Therefore, the mode through which plants interact with already existing elements of a plant community is crucial for the further characterisation of the mechanisms behind the term "*priority effects*". Within this work the mechanisms of priority effects are considered similar to the facilitation/tolerance/inhibition model of Connell & Slatyer (1977a). They characterize the influence of an already established community element on that of a colonizing element either to be facilitative (positive), inhibitive (negative) or neutral. Species which occupy similar niches and thus exert strong ecological competition for similar resources do more likely follow the principle of competitive exclusion (Hardin 1960).

Tan *et al.* (2012a) used phylogenetic relatedness as a surrogate for niche similarity (sensu "phylogenetic niche conservatism") of species in artificial bacterial communities and showed the positive relationships between phylogenetic diversity, priority effects and ecosystem function exist (for details see *Experiments on priority effects*). Positive and/or negative interactions between plants can in turn influence whether priority effects occur and how they affect community structure. Verbruggen *et al.* (2013) point out in a recent review on mycorrhizal fungal establishment in agricultural soils that the outcome of interactions contributing to priority effects might be heavily contingent upon whether interactions between species arriving at different times are symmetrical. Hence who arrives first even becomes more important in face of the actual outcome of interactions between species pairs.

Beyond these rather direct interactions, priority effects may also work indirectly via the plant-soil feedback, either influencing the chemical composition of a site in favour of their own offspring (e.g. in

case of *Eucalyptus regnans*) or by having profound impact on the soil microbial community. This even is possible beyond the total lifetime of the plants inhabiting a site when decomposing plant material is being mineralized and these substances exert effects influencing plant growth and/or establishment of other species (Facelli & Facelli 1993; Ladd & Facelli 2008b) and thus could be the reason for successful invasion or colonisation of species during assembly (Walker, Walker & Hobbs 2007). One example is a study by Hughes & Denslow (2005) on the effects of a non-native, N2-fixing shrub in Hawaiian wetland systems. They could show that due to the invasion of *Falcataria moluccana,* biogeochemical cycling (mineralisation rates) and community dynamics (species abundances) were persistently altered, even facilitating the establishment of other non-native species into the system.

1.1.2 Filter theory

Another relevant theory for community assembly that is related to priority effects is filter theory. Here community assembly is strongly influenced by species interactions and environmental constraints, so called "filters" that allow certain species to establish at a site and others not (Kelt, Taper & Meserve 1995; Hobbs & Norton 2004). These filters determine the conditions by which species are able to fulfil the requirements to establish themselves, to persist and to propagate under the present constraints of biotic and abiotic conditions. Only those species that can establish reproducing populations under the current abiotic conditions are able to pass the "filter mesh" (Kelt, Taper & Meserve 1995). When new land opens, these filters determine which species arrive when and whether they are able to establish a founder community at the new habitat (and hence, being able to exert priority effects on following species).

Positive and negative interactions between organisms (biotic filters) can in turn trigger priority effects and also their intensity. They can be the reason for successful invasion or colonisation of species in cleared or degraded areas (Walker *et al.* 2007). In this sense, priority effects resemble one of the shapes the biotic filter may take. This is the case if one species affects the establishment of another species (which is in the state of "arriving") at a given site/habitat.

Habitats are often initially dominated by fast growing species adapted to the actual conditions (Grime 1974). In time, these species are then substituted by species with more competitive ability thus creating a sequence of arrival and extinction within the assembly process. The filter concept in ecology is useful to investigate whether abiotic or biotic factors are driving the community more strongly if one succeeds to disentangle the relative effects of biotic and abiotic filters determining the realized species composition at a given time. Often, it cannot however give insights into the effects of migration history or the outcome of species interactions. But what we observe in terms of which species are extant at a site is actually the outcome of both abiotic and biotic filters acting simultaneously as well as the establishment and local extinction of a species population in time and space at a given site. Filter effects are considered important mechanisms affecting community assembly and hence ecosystem processes and services.

1.1.3 Biodiversity, plant-plant interactions and effects on ecosystem functioning

Since biodiversity both, affects and responds to ecosystem properties, disentangling the feedbacks and processes involved is vital for understanding the forces which structure communities (Hooper *et al.* 2005). Over the past two decades, with accelerating global change and species loss, the question of what effects diversity itself has on ecosystems has become more important for many ecologists. Biodiversity research is now focusing more on how diversity as an explanatory variable affects productivity (or in general ecosystem function) rather than as a response variable. Hence, Biodiversity-ecosystem functioning experiments became a major part of ecological research.

We know that currently species are being lost at a rate unprecedented in human history (Loreau *et al.* 2001; Balvanera *et al.* 2006) and this emphasizes the need to maintain and restore biodiversity at ecosystem level and to counteract negative effects of biodiversity loss (Hooper *et al.* 2012; Isbell *et al.* 2015). Positive effects of high plant species richness on community functioning have been reported in numerous experiments, with the main outcome being that higher diversity (both in terms of plant species richness and -functional richness) often leads to higher aboveground and (with a time lag) belowground

productivity as well as improved nutrient- and water cycling. Additional findings include positive effects on stability and disease risk (Hättenschwiler, Tiunov & Scheu 2005; Keesing, Holt & Ostfeld 2006; Tilman, Reich & Knops, Johannes M. H. 2006).

The link between plant diversity and (above ground-) productivity is being heavily discussed among scientists, and started with Grime´s concept about the shape of the relationship between diversity and productivity (HBM, sensu Grime1979). His concept states that in highly productive sites plant diversity is limited by interspecific competition whereas in low productivity sites limited resource availability (and/or high levels of disturbance) functions as a restriction towards species richness. At intermediate levels of productivity Grime proposed species richness to be highest, controlled by a combination of both the before mentioned extremes. After several studies in different ecosystems there is still uncertainty about whether Grime´s concept is a generalizable model, which holds for the majority of ecosystems and under different environmental conditions or it is just applicable to only certain sites (see e.g. Fraser *et al.* (2014) for a recent review). Particularly in grassland ecosystems productivity seems to be positively correlated to plant diversity (Hector 1999; Schläpfer & Schmid 1999; Roy, Saugier & Mooney 2001; Tilman *et al.* 2001) but factors that co-vary and thus modulate diversity and hence the effects of diversity on ecosystem function need to be explored further (Hooper *et al.* 2005).

An important concept within the theoretical framework of biodiversity research, explaining the positive effects of plant diversity on productivity is the so called "Complementarity Effect". In many biodiversity-ecosystem functioning experiments niche complementarity between species varying in traits is thought to lead to better overall resource-use at community level, and that particular combinations of functional groups (particularly N_2-fixers combined with grasses) as well as species richness can drive positive diversity effects (Hooper & Dukes 2004a; Kirwan *et al.* 2007b; Oelmann *et al.* 2007b; Temperton *et al.* 2007b; Marquard *et al.* 2009c; Finn *et al.* 2013; Zuppinger-Dingley *et al.* 2014). It may be that this complementarity between different functional groups is a driver of assembly over time, and hence composition, stability and functioning of communities (Hooper & Vitousek 1998; Craine *et al.* 2003;

Young, Petersen & Clary 2005; Gillhaussen *et al.* 2014a) but there are not many studies addressing complementarity in the context of assembly.

Some PFTs may even take the role of ecosystem engineers by improving the establishment and survival of other species or even promoting their growth. N_2-fixing legumes in particular introduce extra N into soils and hence can significantly drive N cycling and community productivity due to their ability to increase N availability either via N sparing or via N transfer. N sparing occurs when a legume uses lesser resources from the soil N-pool when relying more on atmospheric nitrogen fixation and therefore leaving more nitrogen for other members of the community. N transfer occurs when any legume N is being mineralized and reenters the N cycle, via decomposition, exudation or direct transfer via mycorrhizae. There are few ecological studies that have addressed the different possible mechanisms of N transfer, instead the main focus has been on the relative role of the two main aspects of N facilitation: N transfer and N sparing as well as species-identity effects on the interaction outcome across environmental gradients (Brooker & Callaway 2009; Temperton *et al.* 2012b). A few of studies have explored the relative strength of N sparing versus N transfer and found that N sparing seems to play a key role in the shorter term (Carlsson & Huss-Danell 2003; Carlsson *et al.* 2009a). Over the longer term, under field conditions, however, as aboveground biomass is removed as hay in mown grasslands, there is evidence that N transfer may play an important role as well as N sparing during N facilitation (Temperton *et al.* 2007b; Gubsch *et al.* 2011).

These results agree with the stress gradient hypothesis (Bertness & Callaway 1994). This hypothesis states that positive interactions should increase as one moves from benign to harsh environments. Especially in harsh environments (e.g. low initial nutrient content or high environmental stress) legumes may have a positive effect not only on productivity (Maestre *et al.* 2009) but also on assembly processes. This would be the case, when allowing certain species to pass through the *filters* due to specific and positive interactions with already present species, without which it would not manage to establish. An example is when a nurse plant enables another species to establish under harsher (climatic) conditions that it is normally accustomed and hence allowing it to survive and adapt to this environment (Valiente-Banuet *et*

al. 2006) or (in the case of a legume) by increasing the probability of establishment for species which require higher N concentrations in the soil (Rascher *et al.* 2012). In such a case (biotic-) facilitation increases establishment of species who would have been assorted by the abiotic filter elsewhere.

1.1.4 Priority effects and implications for restoration ecology

Central European grasslands belong to the world's most species rich ecosystems on small scales (Wilson *et al.* 2012). They require management to survive (mowing, grazing) but land managers are increasingly giving up on managing especially marginal sites. These areas usually are un-feasible for land managers when the effort (regardless whether it is monetary effort or workload) exceeds the benefit. This together with agricultural intensification pose some of the major threats to extensively managed, species-rich grasslands (Temperton *et al.* 2012a).

Given the background of restoration ecology, the reestablishment of ecosystem services on degraded land is of great interest (Hobbs & Harris 2001). Therefore, community assembly is a key issue related to restoration dynamics (Temperton 2004). To regain the ability of a once established systems to sustain ecosystem services over a long period of time is a main goal of restoration. Thus a high diversity, self-sustaining semi-natural ecosystem with the potential of delivering profitable feedstock with low effort is of high interest for restoration approaches. By finding a way to increase diversity and productivity at the same time win-win situations between land owners, conservationists and the increasing demand for food and renewable energy can be achieved (Tilman *et al.* 1997; Isselstein, Jeangros & Pavlu 2005a; Tilman, Hill & Lehman 2006; Tilman *et al.* 2006; Bullock, Pywell & Walker 2007b; Tilman *et al.* 2009; Weigelt *et al.* 2009). If a change in the prioritisation of restoration attempts would evolve, which would aim towards sites unsuited for intensive agriculture (e.g. marginal sites) as well as towards restoring biodiversity, this win-win situation could become reality and thereby counteract biodiversity loss and environmental degradation (Rey Benayas & Bullock 2012).

A study by Fukami *et al.* (2005) introduced initial species composition (under certain constraints) as a potential way of fostering priority effects through increasing the hypothetical number of species interactions by increasing diversity within the starting community, hence the influence of initial (sown) diversity became subject in our own further experiments and investigations (and therefore was included as a factor into my experiments; **see Manuscript 4**).

In a slightly different context, also other groups found positive effects of sowing high diversity mixtures in applied research during restoration attempts in old fields (Dedov *et al.* 2006; Bullock *et al.* 2007b; Bullock *et al.* 2007b; Leps *et al.* 2007; Fagan *et al.* 2008). First to mention is a study by Bullock *et al.* (2001b) where he observed positive effects of sown plant species diversity on productivity among seven sites in a restoration experiment and found that the increase in hay yield was on average 60% compared to low diversity plots. In 2007b they could show, that sowing high diversity mixtures increases both, diversity and productivity still detectable after eight years and in two different sites in southern England. They proposed that the variation in hay yield was related to differences in the number of non-leguminous forbs, therefore increased hay yield was an effect of the greater range of life forms within plants contained in the high diversity mixture, rather than a simple fertilizing effect of legumes (see paragraph on *Biodiversity, plant-plant interactions and effects on ecosystem functioning*).

Evolving models of succession, assembly and state-transitions are at the heart of both community ecology and ecological restoration (Young *et al.* 2005). Testing how we can use priority effects for improving diversity as well as community function is an exciting field of research with direct relevance for restoration and management. As previously discussed, time and order of arrival of species can have profound effects on the long-term trajectories of communities (Hoelzle, Jonas & Paschke 2012; Vannette & Fukami 2014). From the very first start of the debate on how communities assemble, priority effects were considered a crucial theoretical element within that concept, and are currently being explored as potential management tool in ecological restoration (Temperton 2004; Martin & Wilsey 2014; Wilsey, Barber & Martin 2015; Young *et al.* 2015).

Recently, studies showed that priority effects could be used to successfully assist the establishment of less-competitive species in communities (Porensky, Vaughn & Young 2012; Cale *et al.* 2013). A number of experimental studies on perennial herbaceous plant communities in California have shown that already a few weeks of differences in arrival time can significantly affect community structure (Abraham, Corbin & D'Antonio 2009; Grman & Suding 2010a; Stevens & Fehmi 2011; Dickson, Hopwood & Wilsey 2012; Cleland, Esch & McKinney 2015). And that these priority effects in natural communities also depend on species specific germination time.

In a further study, already small priority effects of native perennial grasses over exotic annual grasses resulted in substantially greater cover by the natives (Vaughn and Young 2015 *in press.*). That means, priority effects may be particularly relevant for testing the mechanisms underlying the competitive advantage of (invasive) annual plants over (native) perennials (Young *et al.* 2015). It has been stated and confirmed that the competitive advantages of annuals over perennials are driven by the earlier germination and initially higher growth rates of the annuals (Harmon & Stamp 2002; Verdú & Traveset 2005; Lulow 2006; Grman & Suding 2010a; Cleland *et al.* 2015). However, most of these studies were carried out at a single site and in a single planting year. In consequence, it cannot be concluded from this on the general strength and consequences of priority effects. .

1.2 Ecological experiments

Experiments, especially in ecology, are heuristic tools which aim to advance our knowledge on the basis of what we know (Beierkuhnlein & Nesshöver 2006) but at the same time they need to reduce the complexity of an observed system to the variables of interest (possibly without neglecting relevant properties). Since our knowledge on natural systems and their underlying processes is considerable but yet limited and much of the knowledge about these systems has been created through observational studies, ecological experiments bear the challenge to adequately display the real spatio-temporal heterogeneity of ecological objects (and all relevant interactions) and to test mechanisms within the limits of our current

knowledge. Into this context, a quote coming from Tansley (1935) fits well, saying: "*Actually the systems we isolate mentally are not only included as parts of larger ones, but they also overlap, interlock and interact with one another. The isolation is partly artificial, but it is the only possible way we can proceed*".

Experimental approaches are required for mechanistic explanations of ecological phenomena (Agrawal *et al.* 2007). The quality of an experimental approach, however, depends on whether the effective variables are considered (Hurlbert 1984). This may lead researchers to focus their attention on processes and/or observations which they consider to be of importance but in fact they are not the key drivers of a system. We aim to go beyond of what we know and apply treatments which we hypothesise on behalf of previous insights and our resulting understanding. However, in the design of ecological experiments one must be aware of the level of abstraction an experiment is being carried out on as well as the effect of the treatments on any of the inherent elements of the system, to not (unintentionally) make mistakes which may lead to "hidden treatments" (Huston 1997), or the misinterpretation data (Grime 1998; Loreau 1998).

In "artificial" experiments (e.g. greenhouse- or lab experiments) therefore simplification is the principal approach through which we are trying to concentrate on specific and/or isolated processes of relevance and gain further insights. This works through partially excluding "unknown" or undesired interactions thus reducing the noise of our own callowness, aiming to create universally applicable laws (Cartwright 1983).

In contrast, field experiments comprise these momenta of natural interactions of unknown quality to us, forming a different approach from the "isolated", simpler experiments in the lab or greenhouse. By exposing an experimental setup to natural conditions we allow for real-world interactions and make a real-life benchmark with what has (optimally) been found before and therefore account for the complexity of natural conditions. However, we generally do not know (especially up to the initial time point a field experiment is assembled) about the multitude and magnitude of effects interfering/interacting with our experimental manipulations. We have to be aware of the fact, that such field experiments – through the

rather narrow framework in which they operate (e.g. specific soil conditions or environmental background) only work within these boundaries and results obtained can often only be interpreted under these specific conditions. In that sense, field experiments remain case studies (Norby & Luo 2004) that allow taking real-life, natural conditions into account but only deliver results for a rather narrow set of conditions.

Overall, experiments enable to manipulate elements within a "natural" framework and enable to falsify hypothesis in a way which is impossible with pure observational studies. Worth mentioning is the fact that experiments allow to ask context specific questions which a proper design is allowed to answer. These answers then are of a quality which is rarely found by pure observational studies. Treatments can be applied and directly referenced to "untreated" control conditions thus acquiring much higher precision and quality of results (by being able to increase the certainty of our findings by increasing replication) compared to observational studies.

1.2.1 Experiments on priority effects and effects on community function

1.2.1.1 Priority effects in model ecosystems

The question to what extent community assembly or more precisely assembly history affects ecosystem function and what particular processes stand behind the term "priority effects" has led to a recent revival of this topic popular among theoretical ecologists from the beginning of the last century. Fukami (2004) created a lab experiment in microcosms using different uni-cellular organisms such as protists and rotifers to artificially alter ecosystem size and assembly history and monitored assembling communities for 50-100 generations. The results showed that history affected diversity more strongly in smaller ecosystems, presumably owing to greater priority effects that occurred here. He further argued that, because species immigration is essentially stochastic, ecosystem size is variable and priority effects can have a strong impact, possibly also shaping size-diversity patterns in natural communities.

As mentioned above, the debate on whether communities are restricted in their development by environmental forces or assembly history is still ongoing. Fukami *et al.* (2005) contributing to this discussion, described a grassland field experiment in which they influenced initial species composition by sowing different diversity seed mixes (zero, four and fifteen species) and allowed for subsequent natural colonisation to see if communities converge in their species composition and traits due to environmental drivers. They found out that the answer depended on the level of community organisation, since initial differences in species composition were still affecting community composition after 9 years but species' traits converged. The bottom line was that the simultaneous operation of species-level priority effects and trait-based assembly rules drove community assembly, confirming both sides in the debate (since species turnover was highest in unsown plots). This experiment inspired the study performed in **Manuscript 1** insofar that the initial species composition was influenced but natural colonisation was allowed (as also was the case in the Priority Effect Experiment described below). In this study in the Habitat Garden Assembly Experiment in Jülich, we tested whether sowing two differently diverse seed mixtures often used in the restoration of dry acidic grassland would create any priority effects over time. We found that the sowing event was still detectable in the community after four years in terms of aboveground biomass as well as the proportions of plant functional groups present, but that species richness varied strongly each year. Thus we found a priority effect but not for all parameters studied.

As experiments on the assembly of plant communities are usually constrained by the relatively slow turnover and generation times inherent to the system, some theoretical ecologists tend to use more simplified systems by studying microbial communities in constrained environments such as microcosms. Although results from these studies may not be directly be transferable to higher organisms, these studies can provide interesting insights and pointers for what to focus on in higher organism interactions during assembly. In a study by Fukami *et al.* (2010) the effects of a small variation in assembly history during early community assembly of wood-decaying fungi exerted strong variation on community structure and as a result also on function (carbon dynamics), attributable to the outcome of competitive and facilitative

interactions during assembly. This points out that the outcome of priority effects on the individual species level could be translated also to higher levels of ecological organisation.

This latter aspect was confirmed by another study which was carried out on wood inhabiting fungi, but under natural levels of environmental variation, showing that the effects of assembly history (order of species arrival) on species population dynamics and thus community structure consistently transfer to ecosystem level processes such as nutrient cycling (carbon, nitrogen and decomposition (Dickie *et al.* 2012). Nevertheless, a recent study suggests that consequences of priority effects for species coexistence requires explicit consideration of environmental variability (Tucker & Fukami 2014).

The strength of competitive interactions during priority effects possibly resulting in an exclusion of later arriving species could experimentally be related to the phylogenetic relatedness of observed species pairs in another microbial study working with yeast communities (Peay, Belisle & Fukami 2012). Their study showed that competition between closer relatives was more intense owing to higher ecological similarity, consistent with Darwin's naturalization hypothesis.

Tan et al. (2012) also used phylogenetic relatedness as a surrogate for niche similarity of species in a similar experiment on microbial communities and showed the positive relationships between phylogenetic diversity, priority effects and ecosystem function exist, highlighting the importance of priority effects for understanding the links between species diversity and ecosystem function. Despite these studies being performed on microbial communities we hypothesised priority effects in plant communities to be stronger between species with the same phylogenetic background but on the other hand that complementarity between species should be stronger if the phylogenetic relatedness is lower. Therefore we chose to work rather with plant functional types (species groups categorized by their differences in traits) since within the community of biodiversity ecologists there is a consensus that not diversity per se -but functional diversity contribute more to the positive effects of biodiversity on ecosystem level (Diaz & Cabido 2001). Thus we were particularly interested in varying the arrival order of plant functional types to increase productivity by niche complementarity over time.

1.2.1.2 Priority effects: Experiments with plant communities

Ejrnaes, Bruun and Graae (2006a) manipulated timing of arrival of pre-selected species groups ("specialists" and "generalists") , fertility, soil and disturbance in grassland microcosms and found that species composition was mainly influenced by timing of arrival but also that "the probability for multiple equilibria appeared to increase with productivity and environmental stability". These findings propose historical contingency to be more influential even if the influence of environmental factors on species richness and invasibility was strong.

Koerner et al. (2007a) found strong priority effects of sowing legumes before other plant functional types for both aboveground and belowground community productivity in a pot experiment (partially) under natural conditions. They could show that species composition, dominance structure and also productivity were significantly regulated by planting one functional group ahead of the others. This effect was still detectable after two growing seasons and four cuttings.

Also manipulating the arrival order of plant functional types in a grassland mesocosm experiment, Kardol et al. (2013b) found that timing of seed arrival affected plant community divergence and leaf chemistry but not community productivity or gas exchange and that the effects of timing of arrival were stronger on more fertile soils, possibly because of increased growth and hence asymmetric competition exerted by the plants sown first.

2. Own contribution within research on priority effects

2.1 A ladder of experiments

As mentioned above, ecological experiments serve a heuristic purpose. They allow the experimenter to make empirical observations, to test hypotheses and to infer or to induce principles coming from smaller systems and to convert findings into theoretical frameworks. By this, one might either be able to form a new theory or to prove/confirm an already existing theory. The scale (time and space) in which an

experiment takes place specifies the level of abstraction on which results can be interpreted. For example, results stemming from a greenhouse experiment have limited generalizability but can often elucidate mechanisms more directly/easily than field experiments. To be able to deduct the mechanisms and understand how priority effects function in and on ecosystems we therefore chose an approach in which we started a set of three experiments, ranging from greenhouse to the field addressing priority effects. We created a ladder of experiments, where we started off with a (reductionist) greenhouse experiment, going over an intermediate microcosm experiment under natural climate conditions, to a rather comprehensive field experiment.

a) The greenhouse experiment

This first experiment served principle of reductionism and was set up for the purpose to test what had been previously found (e.g. Körner et al. (2007a), Kardol et al. (2013b)). Thus, in contrast to preceding studies that tested priority effects of different PFTs by sowing one PFT five weeks ahead of the others this experiment was extended by the factors "Seeding density" and "Sowing interval" as factors possibly interacting with priority effects. We confirmed the findings of Körner et al. (2008) showing an increased aboveground community biomass production when legumes were sown first but we also found that the priority effects of sowing one PFT first was larger in effect size than any density or sowing interval treatments. The main mechanism behind the observed priority effect was considered to be size asymmetric competition. This is promoting the PFT sown first in respect to later sown PFTs, as well as smaller rooting systems of legumes allowing better root foraging of the later arriving PFTS in the legume-first treatment. We could not test this mechanism in this study as we did not measure belowground productivity. Further details on the rationale behind this experiment and its results can be read in **Manuscript 3**.

Fig. 2: Experimental communities consisting of 28 typical central European grassland species (Forbs: 14, Legumes: 7, Grasses: 7) grown in pots with sown densities of 1,5, 2,5 and 5 g/m2, crossed with PFT order of arrival (Forbs, Legumes and Grasses) treatment and an intervall of either 3 or 6 weeks between sowing events.

b) Mesocosm experiment

In 2011 we initiated two similar priority effect experiments at two sites across Germany. One was a microcosm experiment in large containers (1m x 1m x 1m) filled with top-soil collected on an old field site in western Germany. Order of arrival of three different plant functional types (legumes, grasses and non-legume forbs) was varied with different intervals (2, 4 or 6 weeks) between sowing the first and subsequent PFTs. Additionally these treatments were fully crossed within two diversity levels (high: 28 species and low: 8 species diversity) and plots were randomized within the setup. Priority effects were found in the first year of sowing forbs before the other groups, but this effect changed over time, possibly due to cutting/mowing and was not as strong as the legume-first effect in the greenhouse experiment (see Fig. 2). Response parameters were species specific cover as well as community biomass, additionally plots were cut twice a year (in June and September) and biomass was removed from the plots (see Temperton *et al*. book

chapter in Foundations of Restoration Ecology Island Press submitted). Intense April drought impeding germination of sown seeds as well as competition mainly from forbs coming up from the seedbank (weedy arable species) were the reasons for the deviating results compared to other, similar experiments. Nevertheless, we decided to keep up the experiment to use it as a testing platform for methods (e.g. ingrowth cores which were later used in the Priority Effect field experiment or transferring the Optode technique to field conditions) as well as following effects of diversity on community productivity over time (until June 2013). Although, this experiment did not yield any further insights on the mechanisms through which priority effects regulate community function in exchange with climate or plant diversity, it brought up valuable points (together with the Bernburg field experiment) and practice to be addressed and used in later experiments (results will be published as part of a book chapter by Temperton, von Gillhaussen, Baasch and Kirmer, "Timing is everything? Linking biodiversity & ecosystem functioning with assembly for restoration practice" in Foundations of Restoration Ecology 2nd edition, Island Press, submitted)

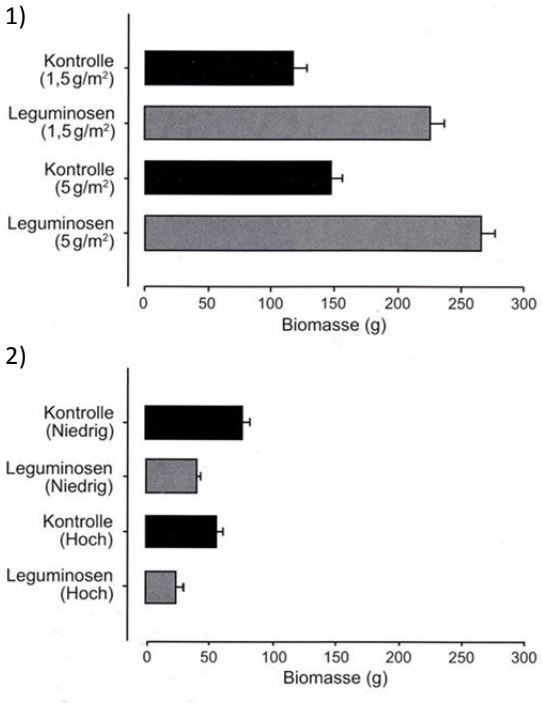

Fig. 3: Contrasting results of sowing legumes first. Data originating from 1) the greenhouse experiment and 2) the mesocosm experiment (in photo). Graphs taken from Temperton (2012).

c) The Bernburg Field Experiment

In 2011, the lab of Professor Sabine Tischew in eastern Germany (in collaboration) also set up a field experiment to test priority effects with a similar approach on their much sandier soils in Bernburg. In this context we investigated the suitability of seed addition with varying PFT sowing sequence to restore degraded ecosystems and to improve ecosystem function at the same time. Apart from exporting the original idea into the field, we teste the step-wise ("mechanistic") seeding approach previously already applied in the other experiments against a simpler sowing technique ("restoration") where the whole seed mixture was sown after the initial sowing of one PFT, The restoration approach considered easier to handle by restoration practitioners and farmers (see Fig. 5). Response parameters were species specific cover as well as community biomass, additionally plots were mown twice a year and biomass was removed from the plots (in June and September, according to agricultural practise). Unfortunately this experiment which started in the same year as the mesocosm experiment experienced the same complications. The early onset of drought and the emergence of weedy species from the seedbank delayed and overshadowed germination of sown target species and possible priority effects. We did find however, that the longest sowing interval showed a tendency towards a priority effect. In addition it was interesting to note that contrary to other studies we did not find that priority effects were stronger in this less-nutrient rich environment than say the Priority Effect field experiment in Jülich. This is discussed in the book chapter by Temperton (2012).

Fig. 4: Fieldsite in Bernburg (Saxony-Anhalt, Germany) with experiemntal manipulations investigating different seed addition techniques to make use of priority effects for restoration (Foto: Anita Kirmer).

2.2 The Priority Effect Experiment

In 2012 we established a large scale field experiment to test effects of varying arrival order of PFTs on community functioning under natural conditions. With our knowledge from previous experiments, we aimed to create a system that provides ecosystem services (biodiversity, productivity) with low to zero input regarding management intensity on one hand but with the ability to deliver usable feedstock for example for bioenergy conversion on the other hand.

According to Chase (2003b), if experimentally tested, priority effects and thereby the evidence for their ability to create multiple stable equilibria in communities requests three conditions to be met: (1) The initial abiotic conditions must be identical (which is never the case in natural systems) and well known. (2) Many replicates of communities where only the sequence of colonization is varied must be studied, and (3) a long enough time period for communities to approach some sort of equilibrium or limit cycle. Additionally, for studies running on a landscape scale (or observations in natural systems), all species from the regional species pool need to have repeated access to the observed community.

Taking this into account we set up a fully crossed and randomized, factorial experiment located near the Forschungszentrum Juelich GmbH, at an old field site of app. 0,5 ha size. The species composition

consists of typical central European grassland species sown (two diversity levels: 9 and 21 species, according to) on two different soil types (A: *Cambisol*, B: *Anthrosol*). Experimental factors are tested in large plots of 4x4m each with eight replicates per treatment (four on each soil type) and a respective number of control plots as well as monocultures and plots without any sowing. Unlike most biodiversity-ecosystem functioning experiments plots were not weeded after initial sowing and therefore open to assembly of non-target species. Within the experiment we are testing the effects of species richness as well as sowing sequence on the assembly of a semi-natural, sown grassland. Through differences in assembly sequence (varying arrival order of three different plant functional types: *grasses, forbs, legumes;* with a five week interval between sowing events) we aim to influence species composition and dominance structure of the resulting communities in such a way that we are able to use plant-plant interactions (complementarity effects, legume facilitation) to positively influence nutrient use efficiency and thus productivity.

In the year of establishment we measured species specific cover, aboveground biomass production and belowground productivity (in the first 8 weeks of establishment using the in-growth core method). Since long-term studies in (restoration-) ecology are scarce (Vaughn & Young 2010) and often yield the most valuable results (Likens 1989) we conducted this experiment as a long term experiment also to be able to assess positive diversity effects (which are thought to increase with time) and stability, convergence or divergence of resulting alternative stable states. Details on experimental setup, site preparations and results from the first year of establishment can be seen in **Manuscript 4**. To my knowledge, besides some studies observing priority effects on landscape scale and/or in the context of restoration practise and nature conservation (Grman & Suding 2010a; Martin & Wilsey 2014; Wilsey *et al.* 2015; Young *et al.* 2015), this is one of the very first times priority effects and their effects on community assembly have been tested experimentally in the field.

Table 1: Overview on temporal progression and own contribution to the research activities within the Priority Effect Experiment.

Action	Time frame	Own contribution
Project idea (Vicky Temperton, Johannes Max)	2011	Part of discussion, pre-experiments
Further development of research questions and experimental design	2011	Part of discussion, literature research, development of protocols, initial sampling, species and site preparation
Initial setup of the experiment	2012	Preparation, coordination and execution
Data collection and site maintenance	2012- 2014	Coordination and execution
External co-operations with Andreas Burkhard (FZJ), Dr. Nicolai D. Jablonowski (FZJ), Denny Popp (UFZ)	since 2013	Coordination of sample- and data transfer

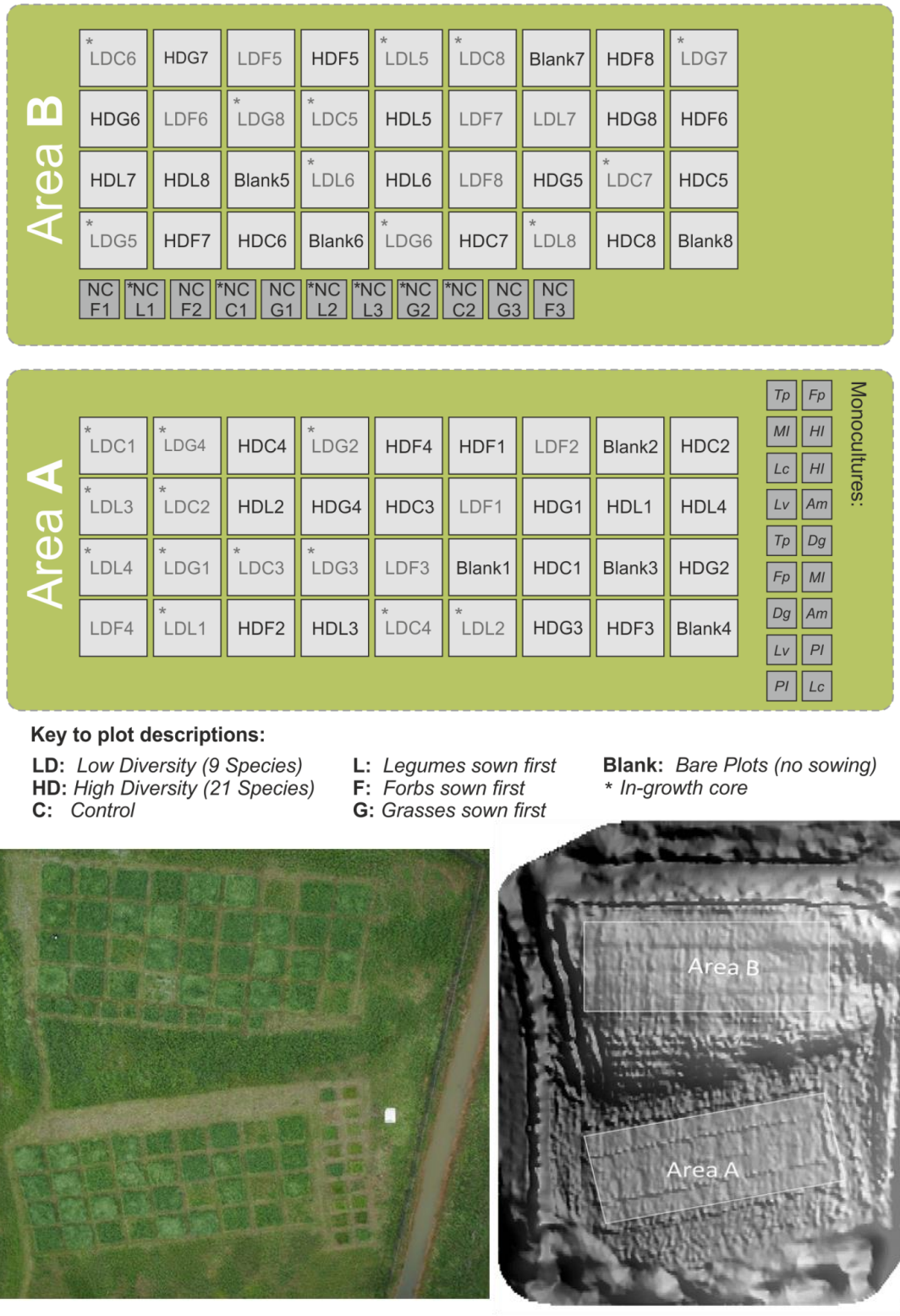

Fig. 5: Schematic representation of the Priority Experiment Jülich (upper image) with plot descriptions reflecting treatments. Arial image (bottom left) and digital elevation model (bottom right) of the Priority Effect Experiment in Jülich, Germany (Foto: Andreas Burkhard).

3. Summarizing Discussion and Conclusion

The prevailing question on which this thesis is based on is, to what extent we are able to use our knowledge on ecological systems to influence (or direct) community assembly in early development stages and guide it towards a desired trajectory or function. More directly: are we able to use priority effects to influence community composition and make further use of biodiversity effects (such as facilitation or complementarity) to improve the quality and quantity of biomass in semi-natural grasslands? If so, this could be very useful during ecological restoration of semi-natural grasslands, a habitat of very high species diversity at small scales that is currently highly endangered by intensification and land abandonment (Temperton 2012; Habel *et al.* 2013).

In sown grasslands, we are able to reduce some of the key driving factors during assembly, such as dispersal limitation which we are obliged to accept in natural systems. Although the assembly processes which mediate between a potential species pool and the realized community (through environmental and biological filters (sensu Kelt *et al.* 1995; Hobbs & Norton 2004) are still persisting, dispersal and microsite limitations usually don´t play much of a role in sown (mesic) grasslands (Münzbergová & Herben 2005).

Manuscript 1 (Plueckers *et al.* 2013a) displays some of these particularities of working on assembly related research questions in semi-natural grassland communities in nutrient poor sites (dry acidic), in the closer context of restoration. Here the role of starting diversity (simulated by two different starting community sizes) was investigated as means to simulate differing dispersal filters which are seen as one intersect between potential species pool (gamma diversity) and realized species pool (alpha diversity). Previous experiments had shown that starting diversity may tremendously affect productivity (Bullock *et al.* 2001b; Bullock *et al.* 2007b; Pywell *et al.* 2007) and other ecosystem functions such as stability or arthropod diversity (Dedov *et al.* 2006; Bezemer. & van der Putten 2007b). Over a period of four consecutive years, the priority effect of different starting diversity caused varying effects on response variables, also with differing persistence over time. While aboveground productivity was still affected by

starting diversity after four years, effects on other response variables like total species number or total cover however were not significant. Although we could show, that community functionality (biomass) was affected by the priority effect (created by a different starting diversity) low establishment of target species was observed (40% in the fourth year). This was mainly attributed to an unsuitability of abiotic niches for the species' sown and the lack of proper regeneration niches for some of the species (microsite limitation). Additionally an explanation for the lack of the treatment effect on many of the measured response parameters could be the low responsiveness of the observed system due to low nutrient availability and thus growth –and thereby- interactions between plants could have been dampened compared to more mesic systems.

However this study lead to substantial insights for future approaches studying priority effects.

1) The species pool selected for the experiment should match the environmental constraints of the system observed.
2) Responsiveness of the system should allow fast assessment of possible effects and mechanisms and the dynamics of the system should be followed over a number of years.
3) The measured response parameters should be selected carefully and should be broad so as to capture possible priority effects.

Already Ejrnaes *et al.* (2006a) and Kardol (2013b) pointed out in their studies on priority effects that the most plausible explanation for the results found is to be the outcome of asymmetric competition between species establishing first and species that try to colonize thereafter. At the same time, they both point at the importance of resource availability for the outcome of this interaction.

Coming from the background of working in nutrient poor (dry acidic) grassland types we therefore considered nutrient availability to be one of the key drivers, shaping the outcome of plant-plat interactions in the context of priority effects. In this context the role of nitrogen fixing legumes became a central focal point in my research and this especially under harsh environmental conditions (*sensu* the stress-gradient hypothesis). Legumes are actively or passively affecting nutrient cycling, leading to a net positive effect

on plant and community performance (Brooker *et al.* 2008) since legumes rely also on atmospheric nitrogen through their association with N_2-fixing rhizobia. Root systems of legumes can be less extensive in biomass and occupied area, leaving space which may grant later arriving species more resources in terms of soil space (niche space) left to explore (Ravenek *et al.* 2014b).

Apart from their reduced demand on soil nitrogen (N-sparing), facilitation could be shown via root exudation or mycorrhizal network linkages (N-transfer) (Paynel, Murray & Cliquet 2001; Govindarajulu *et al.* 2005) or through the mineralisation of N-enriched legume tissue when plant parts die off (Tomm *et al.* 1995) posing a potential benefit to later stages of community development. Especially in systems with low plant-available-nitrogen, N_2-fixation is observed to be highest (and even more when non-legumes are present in the community, sensu Temperton *et al.* (2007b)) resulting in enhanced facilitative effects (Hartwig 1998; Nyfeler *et al.* 2011; Bessler *et al.* 2012).

These positive interactions were subject of my further investigation of possible mechanisms behind priority effects in **Manuscript 2**. Therein we addressed an issue which is however still a main challenge in the field of plant-plant interactions: how to assess plant interactions belowground? In particular I was interested in the quantification of belowground processes in respect to N-facilitation, more precisely finding evidence of increased nitrogen availability for non-legume species when growing together (intercropped) with a legume. Intercropping in agricultural practise has been practiced for a long time as a means of improving nitrogen content and retention in agricultural systems (Horwith 1985; Mariotti *et al.* 2009; Tosti & Thorup-Kristensen 2010). Besides this, it is thought to play a major role in biodiversity effects with many studies addressing this mechanism behind positive biodiversity-productivity-relationships (Shen & Chu 2004).

Especially disentangling the links between N transfer and N sparing N is tricky during experiments. We aimed to see if we could detect any preferential movement of non-legume roots towards those of the legume (or under the legumes roots) which we would expect if N sparing was playing a key role in the interaction. In addition, the critical issue of how to separate the roots of different species is a key

challenge that a number of labs have been working on over the past decade. Possible methods include analysis of root DNA (Mommer *et al.* 2008) or infrared spectroscopy (Roumet *et al.* 2006) as well as using pant species that have been genetically transformed to express green or red fluorescent proteins (Faget *et al.* 2009). We used the latter approach in **MS 2**. Therefore we designed an approach in which we combined different methods, inside climate chambers to be able to visually assess the rhizosphere and distinguish rooting systems of our experimental plants. We planted species mixtures (one legume together with a non-legume, as well as two legume forbs together with a non-legume) and monocultures in rhizotrones (see Fig 7.).

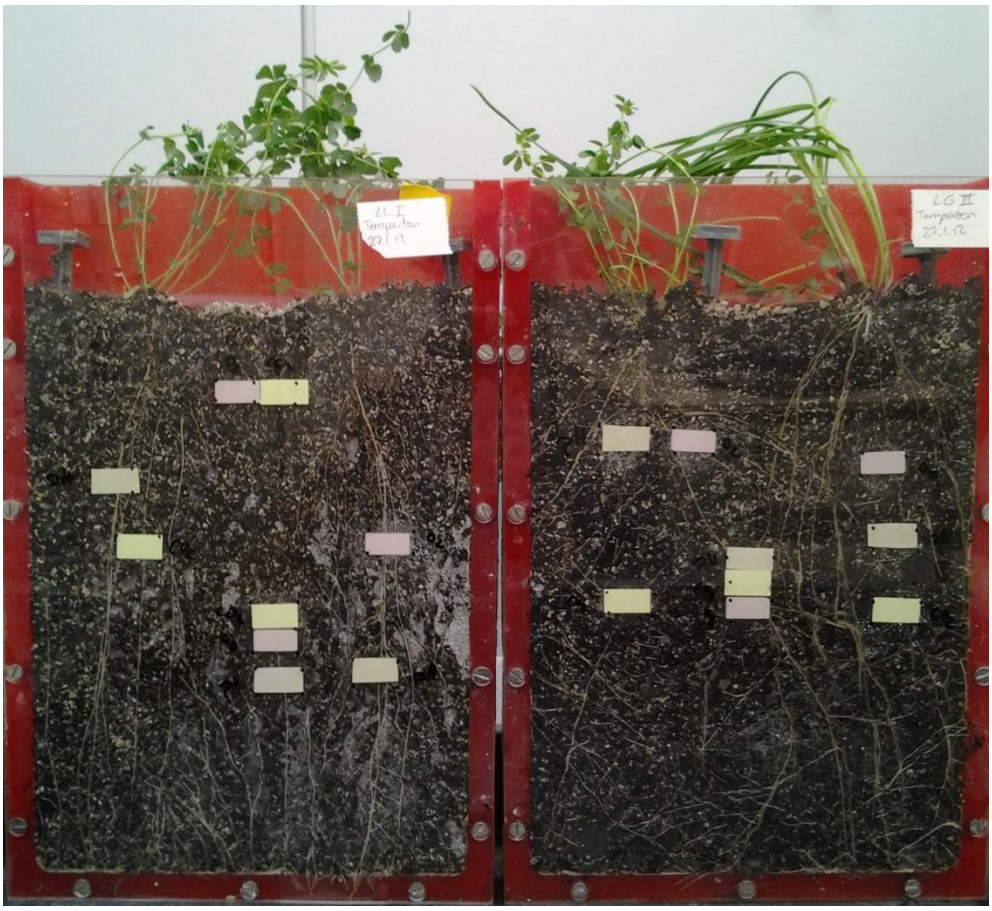

Fig. 6: Example of rhizotrons which have been used to assess root architecture and distribution in the experimental setup of Manuscript 2. Plants grow in a flat, rectangular, pot-like container consisting of a transparent side on which roots are forced to grow along by tilting the container in an angle of approx. 20° towards the transparent side. Shreds on the transparent side show the planar optodes for pH, Co_2 and O_2.

We used genetically modified maize plants (*Zea mays*), which allowed us to visually distinguish the roots of the maize plants by an expression of green fluorescent protein inside the roots, together with non-modified common bean (*Phaseolus vulgaris*). Additionally, we combined this method with planar optodes by which we were able to track pH changes over time, in specific regions of interest (ROIs) where roots of both species did interact and compared this to ROIs without interaction. Although our method succeeded in distinguishing the roots of different species grown in natural soil and following pH dynamics in the rhizosphere of two species over time (what has, never been possible before up to that point), we did not manage to find evidence for N facilitation in the maize when growing with the legume (no higher leaf N values or clear changes in δ15N when growing near the legumes; data not shown in **MS 2**).

The priority effect found by Koerner's assembly experiment (described in chapter "Priority effects: Experiments with plant communities") was strong and statistically sound for different communities varying in species composition (communities were comprised of randomly selected species from a defined species pool). Especially the question of to what extent the observed effect depends on factors, such as community size and density or time interval between the plantings was relevant for assessing the nature of priority effects. Since up to that time we were not able to propose legume facilitation as one of the key drivers of increased productivity in communities where legumes were planted ahead of other PFTs we hypothesized that if N-facilitation was occurring between legumes and non-legumes, the magnitude of a potential positive effect would increase with increasing individual numbers (Marquard *et al.* 2009a). Thus we introduced sowing density into our experiments as a factor possibly correlating with facilitation intensity. At the same time varying density and the time interval between plantings were considered as means to simulate dispersal frequency of natural assemblages. **Manuscript 3** offers an answer to some of these questions, going beyond what Koerner *et. al.* (2007) did. At the same time it opened the stage for another set of questions, extending this research once more.

The next step was to expand our proof of principal from our greenhouse experiments to field conditions since greenhouse experiments and results obtained from there only offer limited transferability when

interactions under natural environmental conditions are allowed (see section on ecological experiments above).

In the Priority Effect Experiment in Jülich, we included additional factors likely to modulate either ecosystem function or modulate the priority effect itself. The factors we also tested were: diversity of starting community (Bullock *et al.* 2001b; Bullock *et al.* 2007b; Leps *et al.* 2007), soil type (van de Voorde, van der Putten & Bezemer 2011; Hendriks *et al.* 2013) and sowing sequence of PFTs (Körner *et al.* 2007a; Kardol *et al.* 2013b) to investigate the effects of the size of the regional species pool (sown starting diversity) together with the interaction of the time of arrival of the different PFTs: legumes, grasses and non-legume forbs. To be able to extent the findings of this experiment beyond the limited constraints of a single field experiment, we conducted our setup on two different soils, thus strengthening the generalization potential of the results obtained and maximizing predictions to a range of varying environmental conditions.

In **Manuscript 4** we assessed community composition and above- and belowground productivity as a proxy for ecosystem functioning during initial assembly within the first growing period in 2012. The main aim of this approach was to evaluate the options of using priority effects for restoration purposes or in (extensive) agricultural production systems as a tool to increase productivity by optimizing nutrient-use whilst at the same time reducing workload intensity. Unlike other biodiversity-ecosystem-functioning experiments we sowed communities in the beginning but then did not weed allowing for natural assembly and interactions with invading species. Based on the results from Bullock *et al.* (2007b) and Körner *et al.* (2007a), biodiversity effects and the interplay of positive interactions among PFTs were hypothesized to increase productivity aboveground whilst reducing it belowground.

Results showed that different biomass allocation patterns between above- and belowground plant parts were found. The dominance of species from the respective PFT sown first (despite the exclusion of aboveground competition by mowing before the second sowing) suggests that there was interplay of two factors during initial community assembly: belowground asymmetric competition leading to aboveground

asymmetric competition. At the end of the first vegetation period we found effects of legumes arriving first on both below- and aboveground productivity.

3.1 Emerging research questions

3.1.1 Alternative stable states and their temporal stability

A topic heavily discussed in ecology are alternative stable states as a consequence of different assembly history (Beisner *et al.* 2003; Jiang *et al.* 2011). The idea behind is that communities at a given site and of a common species pool can be found in one of different possible stable states, with the option to leave this state as a consequence of a shift in parameters, either reflecting environmental perturbations or a change of environmental drivers (Fukami & Nakajima 2011b). The colonization history creates different pathways within community development by affecting the success of later species through priority effects (Shulman *et al.* 1983; Fukami 2004). Research into the assembly of ecological communities has shown that the extant composition of communities is strongly influenced by historical factors and sometimes even has stronger influence than the effects of abiotic conditions on community composition (see chapter *Assembly theory and the importance of priority effects* above).

Martin & Wilsey (2012a) show that alternative (native or exotic species-dominated) states could be created under the same environmental conditions just by altering assembly history in a prairie restoration experiment. It is unclear whether achieved differences in community composition induced by different assembly histories are stable in time (or may even become stronger with time, leading to compositional divergence) or whether compositional differences faint with time as communities with different assembly histories become more similar (compositional convergence).

Fukami & Nakajima (2013) advocate to rather use the term “alternative transient states” than talking of alternative stable states since the latter term implies that community assembly is linear. In fact it is rather a cyclical process frequently being perturbed by disturbances thus he states that a state remains only stable for a limited time. Also explicitly testing priority effects and their contribution to (alpha-, beta- and

gamma-) diversity across different scales and/or along environmental gradients still remains to be done. However, the current model of assembly and succession integrating the theory of alternative stable (or transient) states reflects the most useful approach of community development and assembly dynamics. Still it needs to be tested (for a number of different habitats and/or community types) for generality.

4. Declaration of own contribution to each paper

Concept: Idea for the study and development of experimental design or development of an outline.

Data acquisition: being responsible for organization and execution of data acquisition and doing the measurements together with the help of students and interns

Data analysis: statistical analysis of data and their illustration in tables and figures

Writing: writing the manuscripts, including literature research

Editing: General editing, including providing comments on the storyline and the scope and the science from co-authors.

Manuscript 1:

Sowing different mixtures in dry acidic grassland produced priority effects of varying strength

Christine Plückers, Uwe Rascher, Hanno Scharr, **Philipp von Gillhaussen**, Carl Beierkuhnlein, Vicky M. Temperton

Acta Oecologica-International Journal of Ecology, 53, 110-116. 2013, DOI: 10.1016/j.actao.2013.09.004

Concept: 0%

Data acquisition: 40%

Data analysis: 15%

Writing: 10%

Editing: 20%

(This article is also part of the dissertation of Christine Plückers)

Manuscript 2:

Disentangling who is who during rhizosphere acidification in root interactions: combining fluorescence with optode techniques

Marc Faget, Stephan Blossfeld, **Philipp von Gillhaußen**, Uli Schurr,Vicky M. Temperton

Frontiers in Plant Science, 4, 2013, DOI: 10.3389/fpls.2013.00392

Concept: 20%

Data acquisition: 30%

Data analysis: 10%

Writing: 10%

Editing: 25%

Manuscript 3:

Priority Effects of Time of Arrival of Plant Functional Groups Override Sowing Interval or Density Effects: A Grassland Experiment.

Philipp von Gillhaussen, Uwe Rascher, Nicolai D. Jablonowski, Christine Plückers, Carl Beierkuhnlein, Vicky M. Temperton

PLoS ONE, 9, e86906, 2014, DOI: 10.1371/journal.pone.0086906

Concept: 70%

Data acquisition: 90%

Data analysis: 100%

Writing: 70%

Editing: 70%

Manuscript 4:

Time of arrival of different plant functional types affects above- and belowground productivity in a grassland field experiment

Philipp von Gillhaussen, Uwe Rascher, Nicolai D. Jablonowski, Johannes Max, Carl Beierkuhnlein, Vicky M. Temperton

Submitted to Oikos on May 2015

Concept: 90%

Data acquisition: 90%

Data analysis: 100%

Writing: 75%

Editing: 75%

4.1 Presentation of my work at conferences and invited talks

Conference	Date	Location	Own contribution	Topic
Phytobilanz 2011	February 2011	Germany (Juelich)	poster	Using plant-plant interactions for sustainable bioproduction and restoration in grasslands on marginal land
Salvere Workshop	May 2011	Germany (Bernburg)	poster	Testing priority effects related to sowing high and low diversity and time of arrival of species on assembly and productivity of grasslands on marginal land: first experiments in field and greenhouse
Institute Seminar IBG-2	May 2011	Germany (Juelich)	10' presentation	Using plant-plant interactions for sustainable bioproduction and restoration in grasslands on marginal land
12th annual conference of the European Ecological Federation	September 2011	Avila (Spain)	10' presentation	Effects of species identity and ecological strategy on legume facilitation
INRA Symposium	November 2011	France (Montpellier)	45' presentation (invited talk)	Using plant-plant interactions for sustainable bioproduction in grasslands on marginal land
Evaluation (POF) of the Institute of Bio- & Geoscience 2: Plant Sciences, Forschungszentrum Juelich GmbH	October 2012	Germany (Juelich)	poster	Using ecological and ecophysiological knowledge to increase bioproductivity and nutrient-use efficiency in grasslands
Phytobilanz 2012	November 2012	Germany (Juelich)	10' presentation	Assembly in grasslands
Phytobilanz 2012	November 2012	Germany (Juelich)	poster	Investigating biomass development and distribution in experimental grassland communities influencing assembly history

100th annual conference of the British Ecological Society & Intecol	August 2013	Great Britain (London)	poster	Functional traits and priority effects as a means to stimulate both diversity and productivity in grasslands
Symposium of the department of Botany (University of Basel)	January 2014	Basel (Switzerland)	45' presentation (invited talk)	Priority effects of different plant functional types within grassland assembly: Effects on functionality and diversity from the greenhouse to the field
Symposium of the department of Disturbance Ecology & the department of Biogeography)	March 2014	Germany (Bayreuth)	10' presentation	Invasion in Semi-Natural Grassland: Regulated by "evenness" or presence of dominant species?

4.2 Trainings

Training	Agency	Timeframe
Transferable Skills Training for Doctoral Fellows: Project management, intercultural communication, scientific presentation, scientific writing, career planning	Impuls Plus GmbH	June 2011 - June 2012
Effective Oral Presentation	Principiae	October 2012
Successful scientific writing Block Course	Vicky Temperton	Marc - June 2012

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6. Manuscripts

6.1 Manuscript 1

Sowing different mixtures in dry acidic grassland produced priority effects of varying strength.

Christine Plückers, Uwe Rascher, Hanno Scharr, **Philipp von Gillhaussen**, Carl Beierkuhnlein, Vicky M. Temperton

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Original article

Sowing different mixtures in dry acidic grassland produced priority effects of varying strength

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ABSTRACT

Several longer-term assembly studies on ex-arable land have found that species that arrive first at a disturbed site can play a key role in the further development of the community and that this priority effect influences aboveground productivity, species diversity and stability of the grassland communities that develop. Restoration of nutrient poor, species rich grasslands is often limited by seed dispersal as well as the accessibility of suitable microsites for establishment. Sowing species (i.e. creating priority effects for further assembly) may help overcome such dispersal barriers, but the potential of using priority effects for restoration has not been tested in this type of dry grassland. We tested the hypothesis that sowing two different seed mixtures used for dry acidic grassland restoration onto a sandy substrate (which formed an equivalent to a primary succession) would create priority effects, and that these priority effects would be sustained over a number of years. We followed community assembly and measured aboveground productivity for four years after sowing. We found that priority effects caused by sowing of differently diverse mixtures did also occur in dry acidic grassland habitat, but that how persistent they were over time depended on the response variable considered. Priority effects on species number were not as strong as found in previous ex-arable land studies, whereas priority effects for aboveground productivity were still visible after 4 years. In addition, functional composition of the community still reflected the composition of the seed mixtures 4 years later. Our results suggest that priority effects can occur in nutrient-poor dry acidic grassland but in contrast to more nutrient-rich sites the breadth of responses affected may not be as wide.

1. Introduction

Semi-natural grasslands are hot-spots of European biodiversity with up to 50 species per m^2 (Peet et al., 1983). However, species-rich grasslands are currently threatened by both intensification and land abandonment, which has led to a drastic decrease in area over the last few decades (Kirmer et al., 2012). As such there is an increasing interest in restoring species-rich grasslands, which is often limited by dispersal-limitation (lack of target species propagules), microsite limitation in more nutrient-poor sites and excess nutrient loading of soils (Bakker and Berendse, 1999).

Much is now known about the often positive effects of plant diversity on ecosystem functions from biodiversity-ecosystem functioning experiments (Balvanera et al. 2006), where the diversity gradient is maintained via weeding. In more natural communities, factors other than diversity (such as land management, fertility of soils, climatic conditions or invasive species) are often considered more important key drivers of ecosystem properties (Milchunas and Lauenroth, 1993). However, there are very few studies that have addressed how important diversity effects are in relation to other ecosystem drivers (Flombaum and Sala, 2008; Tylianakis et al. 2008).

There is now increasing evidence that (at least for grasslands) sowing more diverse seed mixtures can create strong priority effects that are detectable in the vegetation long after sowing and that can drive a system as much as land-use history.

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Bezemer and van der Putten (2007) performed an experiment sowing either zero, four or fifteen species of plants onto ex-arable land then followed the dynamics of the system in terms of species turnover, productivity, temporal stability and diversity. In a grassland restoration context, Bullock et al. (2001, 2007) sowed either low or high diversity mixtures using plant mixtures typical for UK grassland restoration on a whole series of ex-arable sites and over a long period of time. Both studies found long-lasting effects (hereafter called priority effects) of initial sowing of seeds on further assembly, in particular when more diverse seed mixtures were sown. Flombaum and Sala (2008) removed species to create a plant species diversity gradient in the Patagonian steppe and found that aboveground net primary production increased with the number of plant species.

The restoration of species-rich communities is becoming a major tool to counteract biodiversity loss but it can also have positive effects on some ecosystem functions, for example increasing biomass production and hence nutrient cycling and reducing erosion (UNEP Nagoya Protocol, 2011). In a world experiencing increasing global change, where historical reference systems often no longer exist, ecological restoration is adapting by focusing as much on ecosystem functioning as on species composition when setting restoration goals (Choi et al., 2008. Hobbs et al., 2009).

1.1. Using priority effects for restoration

Priority effects occur when species that arrive first in an ecosystem significantly affect the further development of the community and thereby strongly influence community composition (Facelli and Facelli, 1993; Fukami et al., 2005). Priority effects can lead to lasting differences in species or functional group dominance, and hence can potentially drive ecosystem properties and functioning. Priority effects can occur on timescales from days to years and can be linked to differences in the arrival time of a species at a site but also to their success in establishing themselves in the community. Priority effects can short-term or lead to alternative stable states in vegetation (Grman and Suding, 2010). As such, restoration projects are often contingent upon priority effects in that they can alter vegetation trajectories as well as impede restoration success in some cases. The mechanisms underlying the priority effects or the timescale upon which they operate are rarely addressed however (Grman and Suding, 2010).

Most of the evidence for priority effects comes from grassland systems with intermediate soil nutrient availability (see Bezemer and van der Putten, 2007; Bullock et al., 2001, 2007). We do not know whether priority effects of sowing different mixtures can play a role in more nutrient-poor dry grasslands. Foster and Dickson (2004) hypothesise that systems with higher resource availability have more available niches but these niches are usually easily filled (packed) with species and this creates more neighborhood competition. In contrast in systems with more limited availability of resources such as soil nutrients, species attempting to establish themselves generally may find more open niche space but mainly face microsite limitation posed by abiotic conditions. Given this, one might expect that sowing differently diverse dry acidic grassland mixtures on sandy substrates (which form an equivalent to a primary succession) would overcome dispersal barriers but that microsite and nutrient limitations may deter establishment of target species more than on a more mesic substrate. The timescale upon which priority effects may operate in dry acidic grasslands may therefore be shorter than for more mesic sites.

Our field study aimed to test the strength of priority effects (in terms of detection of priority effects over time) in a dry acidic grassland created by sowing two different seed mixtures at time zero onto sandy substrate which formed an equivalent to a primary succession and asked the following two questions:

1) Does sowing two different seed mixtures produce priority effects in dry grassland, and how sustainable are they over time?
2) If there are priority effects which traits, processes or characteristics of the ecosystem do they relate to most?

In order to test this, we followed changes in different traits of the community over 4 years: at community level total species number (SN), community cover and aboveground peak biomass as a surrogate of productivity. We also assessed differences between responses of target (desired sown) and non-target species (invaders). At functional group level total species number, community cover and total aboveground productivity were split into functional groups (grasses, legumes, non-legume forbs).

2. Materials and methods

2.1. Experimental design

In autumn 2007 we established a grassland assembly experiment, the Habitat Garden, with two different grassland habitats (dry acidic and mesic grassland). The experimental field plots are on the campus of the Forschungszentrum Jülich, located in Jülich, West Germany (6° 22′0″E, 50° 56′0″N), and consist of 12 plots (randomized, each 2 × 2 m in size and separated by 50 cm paths sown with a non-clonal grass species). The dry acidic grassland plots ($n = 6$) were sown with two different diversity mixtures (whereas the mesic plots were sown with the same diversity of species at the start for removal experiments (not considered here, see also Plückers et al. 2013)).

The dry acidic grassland part of the experiment was designed to follow possible priority effects of sowing differently diverse mixtures on community assembly over time on a sandy substrate which thus formed an equivalent to a primary succession. For each dry acidic grassland plot the original soil was removed by digging out the soil to a depth of 40 cm, and a geomembrane permeable to water and nutrient laid down to avoid root input from plants growing outside the plots and to remove any seed bank. The sandy substrate, which consisted of sand (grain size 0.7–1.4 mm) mixed with one tenth potting soil (with very low nutrient and availability) was then filled into the prepared holes.

We sowed two differently diverse seed mixtures at a density of 3 g/m^2 using typical restoration mixes used to establish dry acidic grasslands in Germany (Rieger Hofmann GmbH Blaufelden, Germany), in December 2007. There were two diversity treatments ($n = 3$ per sowing treatment): S2 consisted of 2 grass and 25 forbs (one of which was a legume), and S7 consisted of 7 grass and 32 forb species (four of which were legumes). Species within the lower diversity S2 treatment formed a subset within the mixtures sown for treatment S7: e.g. the 2 grass species in S2 were also part of the S7 mixtures, the one legume species sown in S2 was also a subset of S7, Both mixture treatments had 12 non-legume forb species in common, whereas S7 has 16 additional different non-legume forb species and S2 has 12 additional different non-legume forb species. These seed mixtures were chosen, to ensure the study had some potential for regional restoration application in the future, such that we chose seed mixtures typically used by restoration practitioners and land managers in central Germany, provided by the wild seed company Rieger Hofmann GmbH. One quarter of each plot was not sown and kept as a control non-sown subplot. The experiment was fenced off to reduce confounding factors such as grazing by deer or wild boar. The plots were mown once a year in

August according to typical mowing regimes for such grasslands in Central Europe and were not fertilized.

Annual precipitation at the site over the 4 years (between 2007 and 2011) was 878 mm, 725 mm, 728 mm, 778 mm, 691 mm respectively (mean 700 mm over 50 years); mean annual temperatures in the same time span were 11,1 °C, 10,5 °C, 10,5 °C, 9,2 °C, 11,2 °C (mean 9.9 °C over 50 years) (measured at the local weather station in the Forschungszentrum Jülich). There were no notable weather extremes (e.g. 100 year climate extremes) during this time, except that mean annual temperature in 2011 was somewhat higher than the long-term mean.

2.2. Plant census and sampling, soil sampling

In the first year (2008) we did not mow to allow initial establishment without disturbance, whereas thereafter hay was cut in late July/early August at peak vegetation. Every year before mowing, a vegetation census assessed cover of every species using a decimal scale based on Braun Blanquet but modified by Londo (1976). Total community cover can therefore sum to more than 100% since the canopy is complex and multilayered. Total aboveground biomass production (dry matter yield, g/m^2) was measured in two 0.1 m^2 quadrats (20 × 50 cm in size) per plot (one randomly positioned at each harvest in the remaining plot and one in the control area). All aboveground plant material per quadrat was cut 2 cm above the soil surface and samples were dried at 70 °C followed by measurement of total community aboveground dry weight. In 2010, 2011 biomass was sorted into three functional groups: legumes, non-legume forbs and grass species. We based these categories on previous functional diversity studies we have performed where these functional groups were shown to perform varying functions within grassland habitats; (e.g. Temperton et al. 2007).

Pooled soil samples were taken per plot once a year in spring or summer (the first two years in spring (March), the last two years in summer (August)) from the topsoil layer (0–15 cm) and analyzed for total C and N content. At time zero (December 2007) one pooled soil sample of soil substrate was analyzed for soil chemistry. For total soil N and C (% weight), the soil samples were dried (12–24 h, 70 °C), ground to a homogenously fine powder and 2–100 mg sample was burned in an elemental analyzer (System: VarioelCube or Leco).

2.3. Statistical analysis

Our experiment is a one factorial experiment testing effects of the factor sowing diversity with two levels of medium and high diversity. Response variables measured were total species number, total cover and total aboveground biomass production per growing season measured at peak biomass in August of each year. However species number, cover and total aboveground biomass production per functional group were also measured, as well as species number and cover of target (sown) and non-target species.

We tested priority effects over 4 years, such that we analyzed most of our data (see Table 1) using Repeated Measure analysis of variance (RMANOVA). Repeated Measure ANOVA was done using univariate procedures with a Sphericity correction to allow for differences in time intervals between measurement dates. All data that did not conform to homogeneity of variance or normal distribution were transformed before analysis: count data (i.e. species number) were square root transformed and all other data multiplied to the power of a specific factor. This specific factor was derived by a power-transformation test to achieve normality of the residuals and homogeneity of variance. This is a useful preprocessing technique and robust against outliers. Back-transformed means and standard errors from the analyses are presented throughout in graphs and tables. All analyses were computed using the Program R. To analyze any treatment difference within single years we used the two-sided student t-test ($p < 0.05$).

Table 1
Results of Repeated Measures ANOVA testing the effect of sowing treatments, time (Year) and their interaction on response variables. Sowing treatment effects show results of testing over the whole time span, whereas Year effects describe how the effect of the sowing treatment factor changed over time. Note this is a summary table of many analyses, such that each line represents a single RMANOVA analysis.

Response variable	Factors					
	Sowing treatment		Year		Year X sowing treatment	
	d.f.	P	d.f.	P	d.f.	P
Total species number	1	0.346^{nsd}	3	0.003**	3	0.2^{nsd}
Forb species number	1	0.238^{nsd}	3	<0.001***	3	0.936^{nsd}
Legume species number	1	0.018*	3	0.004**	3	0.088^{nsd}
Grass species number	1	0.156^{nsd}	3	0.18^{nsd}	3	0.05*
Target species number	1	0.097^{nsd}	3	0.02*	3	0.18^{nsd}
Non-target species number	1	0.29^{nsd}	3	0.002**	3	0.27^{nsd}
Ratio target to non-target species number	1	0.23^{nsd}	3	0.006**	3	0.22^{nsd}
Total cover	1	0.828^{nsd}	3	<0.001***	3	0.11^{nsd}
Forb cover	1	0.02*	3	<0.001***	3	0.081^{nsd}
Legume cover	1	0.005**	3	0.01**	3	0.67^{nsd}
Grass cover	1	0.777^{nsd}	3	0.001***	3	0.28^{nsd}
Target species cover	1	0.019*	3	<0.001***	3	0.032*
Non-target species cover	1	0.186^{nsd}	3	0.004**	3	0.202^{nsd}
Ratio target to non-target species cover	1	0.058	3	0.32^{nsd}	3	0.11^{nsd}
Total biomass	1	0.022*	2	0.024*	2	0.05*
Forb biomass	1	0.0048**	1	0.56^{nsd}	1	0.40^{nsd}
Legume biomass	1	0.007**	1	0.53^{nsd}	1	0.68^{nsd}
Grass biomass	1	0.16^{nsd}	1	0.19^{nsd}	1	0.81^{nsd}
Soil %N	1	0.072	3	<0.001***	3	0.243^{nsd}
Soil %C	1	0.043*	3	0.005**	3	0.51^{nsd}

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; nsd no significant difference.

3. Results

3.1. Is there a priority effect through sowing differently diverse mixtures on diversity and productivity?

Between 2008 and 2011 total species number and total cover of the communities increased over time for both sowing treatments (Fig. 1A and C, Table 1 significant year effect). Sowing different starting diversities affected the total aboveground biomass production and functional group composition and productivity diversity significantly over the whole 4-year time span (Fig. 1B and D and Fig. 2 respectively, Table 1). There was a trend towards total species number and community cover being affected by sowing but only in the first year (t-test $p = 0.056$, Table 1 over 4 years no significant treatment effect).

The number of legume and forb species increased over time in both sowing treatments (Fig. 1B, Table 1 significant year effect). Forb species number was unaffected by sowing treatment whereas there were more legume species in S7 plots in all years (Fig. 1B, Table 1 significant treatment effect). Changes in grass species number were affected by the sowing treatment, with the proportion of grass species over total species increasing in S2 over time (Fig. 1B, Table 1 significant interaction effect)

Cover of forbs, legumes and grasses increased over time (Fig. 1D, Table 1 significant year effect). In all years legumes had a higher cover in the S7 than S2 plots, but a lower cover of non-fixing forb species (Fig. 1D, Table 1 significant treatment effect).

Total aboveground peak biomass (as a surrogate of productivity) increased over time for both sowing treatments (Fig. 2A, Table 1

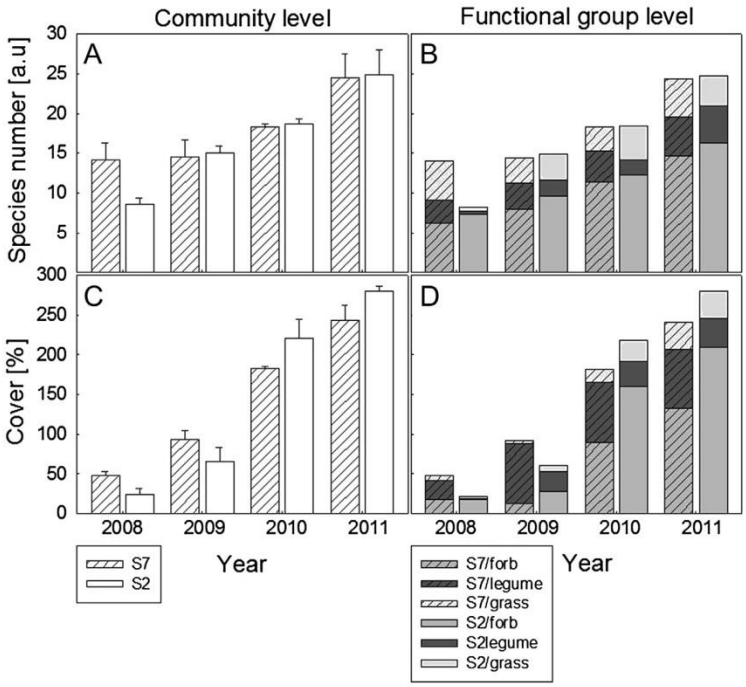

Fig. 1. The development of species number and cover at the community and functional group level. Values are means (+one standard error of the mean) **A and C**: Community level: note that plant cover for a plot can be higher than 100%, because of 3-D aspects of plant community canopies. **B. and D**. Functional group level: non-legume forb species (bottom section), legume species (middle section) and grasses (top section).

significant year effect), but the detailed development of both treatments was different over time, which was confirmed by a significant interaction effect between treatment and year effects (Fig. 2A, Table 1).

The S7 plots had a higher total aboveground biomass production in all years (Fig. 2A, Table 1 significant treatment effect). Total aboveground biomass of legumes was much higher in the S7 plots than in the S2 plots, whereas forbs dominated more in S2 plots (Fig. 2B, Table 1 significant treatment effect). The relative aboveground biomass production of grasses, forbs and legumes did not differ significantly between 2010 and 2011 (when measured). Total aboveground biomass production increased in 2011 in the S7 plots, due to a relative increase in legume biomass (especially of *Lotus corniculatus*). The high variability of the error bars for 2011 biomass can be mainly attributed to the presence of one woody legume shrub in one plot (*Genista tinctoria*).

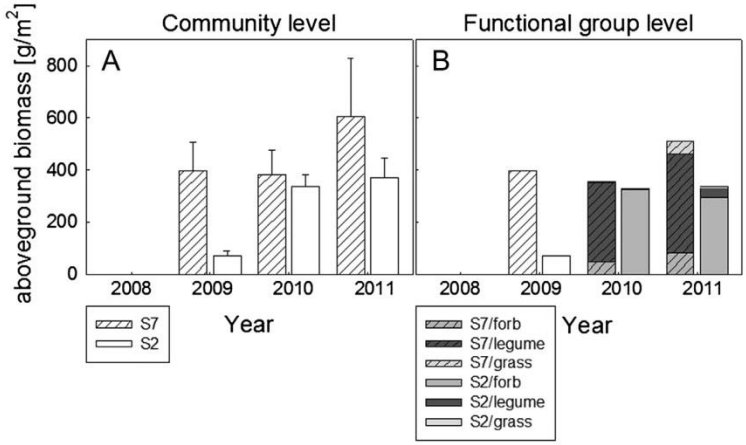

Fig. 2. The development of aboveground biomass at the community and functional group level. Values are means (+one standard error of the mean) **A**. Community level **B**. Functional group level (as in Fig. 3). The total aboveground biomass values of the community level and the sum of the functional group level is not the same because of transformation procedures.

3.2. Is there a priority effect through sowing differently diverse mixtures on establishment of target species and functional groups over time?

Invasion of species increased over time but species richness was not significantly different between treatments (Table 1 see Fig. 3A total number of species). Species number and cover of non-target species (non-sown species) and target species (sown species) of both sowing treatments increased over time (Fig. 3A, Table 1 significant year effect). There was a significant effect of the sowing treatments on the cover of target species (Table 1 significant sowing treatment), but no significant effect of the sowing treatments on the number of non-target species and their cover (see Table 1 no treatment effect overall years). The ratio of target to non-target species number significantly decreased over time as newcomers arrived (Table 1 significant year effect, Table 2 for changes in percentage target species). At the beginning 27 species were sown in the S2 plots and 39 species in the S7 plots. Only 30–40% of the sown species had established after 4 years in 2011 (Table 2), with both sowing treatments having nearly 30% target species in the first year (2008) but had slightly higher establishment in the S2 plots than S7 by 2011 (Table 2). Overall, S7 plots had a higher establishment of target species (relative to non-target species) than S2 plots (Table 2), but the proportion of established target species in the S2 plots significantly increased from 2008 to 2011 (*t*-test $p = 0.050$, data not shown), whereas this was not the case in S7. The non-target species that managed to establish did not mainly derive from the surrounding mesic grassland plots, but were ruderal species such as thistle (*Cirsium*, *Equisetum*, *Rumex* or *Polygonum* sp). Non-target dry acidic grassland species from other sandy plots formed an intermediate group of species that successfully managed to invade at a later time point after sowing. This is as one might expect, given the number of sown species that the treatments shared in common, such that quite a few of the sown dry acidic grassland species in S2 treatments were a subset of the species in S7. In detail, there were 12 "new" non-sown target species that could potentially invade from S2 to S7, versus 24 options from S7 to S2. Our results follow the expected relative success of target species (those sown on a treatment) with high invasion from S7 to S2 than the other way. Over the 4 years, the sowing treatment had a significant effect on assembly. The control plots had significantly less total species number, total cover and total aboveground biomass production over the whole time than the sown plots (data not shown).

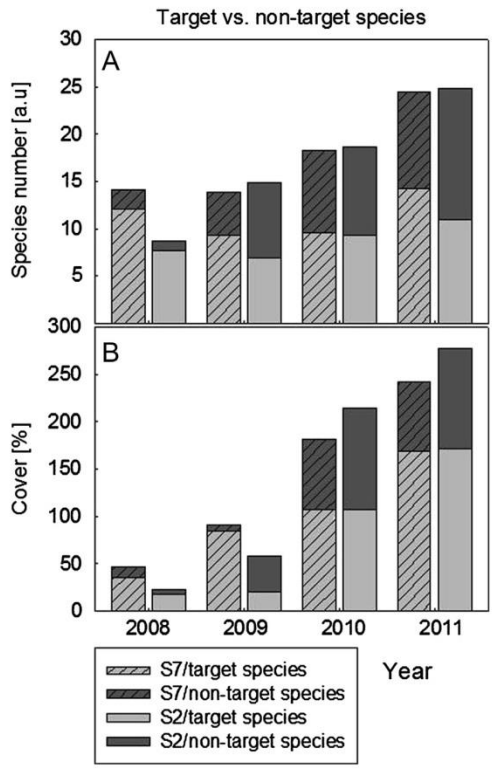

Fig. 3. Sowing treatment effects on the development of mean species number (A) and cover (B) separated into target species (sown species, bottom section) and non-target species (non-sown species, top section).

Table 2
Establishment of target species in the first and fourth growing seasons (2008 and 2011) in relation to the total number of species sown in the original mixtures in 2007. Data show percent values (%) ± standard error.

Sowing treatment	% Establishment of target species	
	2008	2011
S2	28.39 ± 2.5	40.74 ± 3.7
S7	31.62 ± 5.2	36.75 ± 4.3

3.3. Did sowing differently diverse mixtures affect soil conditions?

Sowing different starting diversities significantly positively affected total soil C (% C), with higher soil C in the S7 plots over the whole time span (Table 1 significant treatment effect). Total soil C generally increased, but between 2010 and 2011 it decreased across all plots.

There was a positive trend that sowing different diversities affected total soil N (% N) (see Table 1), with higher soil N in the S7 plots. Total soil N was higher by 2011 compared to the time zero soil samples without species (time zero: 0.005% weight, 2011: S7 plots 0.02% weight and S2 0.01% weight). Total soil N increased until 2010 and then decreased (see Table 1 significant year effect for soil % N).

4. Discussion

4.1. Does sowing differently diverse mixtures produce priority effects in dry grassland, and how sustainable are they over time?

Sowing two different mixtures typically used for restoration of dry acidic grassland in central Europe caused priority effects that influenced community assembly 4 years after sowing. Not all response variables measured, however, still showed evidence of a priority effect after 4 years. The longest lasting priority effects related to aboveground peak biomass, community cover and functional composition of the original seed mixture (whereby the mixture sown was still significantly affecting functional group composition in the extant community after 4 years).

Our priority effects were however not as wide in their effects as those found in experiments on more nutrient-rich ex-arable land (Bullock et al. 2001, 2007; Bezemer and van der Putten, 2007) where initial sowing significantly affected total species number as well as aboveground biomass and stability of the system. Bezemer and van der Putten (2007) could still see differences due to sowing zero, four or fifteen species on ex-arable land after 9 years. In general, these studies and our study indicate that one needs to follow a range of response variables, since the length of time over which a priority effect influences assembly or functioning is clearly

dependent on which variable one measures. It would be interesting to follow more ecosystem functions than just aboveground productivity in further studies, as well as typical vegetation measures. One possible reason for the milder priority effects we found on dry acidic grassland compared to mesic conditions, could be that in communities developing on very sandy substrates, the key limitations to establishment (apart from dispersal) are abiotic in nature (e.g. microsite limitation) and less driven by direct interactions between plant species already present (see Fig. 4). As Foster and Dickson (2004) highlight in their conceptual model of how invasion is modulated by available nutrient resources for plants: systems with higher resource availability experience more niche packing and hence more competition than systems with lower resource availability. Invading species experience lower microsite limitation however, since abiotic conditions are more benign. Sowing more diverse seed mixtures should initially increase the niche packing speed since it removes dispersal limitation, however at later successional stages the area with less seeds sown should allow more new invaders to establish than the high diversity site. In contrast in more nutrient-limited systems, species attempting to establish should generally find more open niche space but mainly face microsite limitations posed by abiotic conditions, as we found in this study. Sowing more diverse mixtures should initially decrease dispersal limitation and increase establishment of target species. In later succession (in contrast to the higher resource scenario) a higher proportion of the established species will be new invaders (non-target) since a lower proportion of the sown species will be able to establish due to microsite limitation. During later succession, facilitation by nurse plants may help new invaders to establish, and the potential for this may increase in more diverse sites.

By sowing two differently diverse seed mixtures on a sandy substrate we influenced the availability of propagules and reduced the dispersal limitation typical of such nutrient-poor grassland communities in this early stage of assembly. We made species available through sowing and gave these species the chance to establish themselves first and thus cause priority effects in further assembly. Considered within filter theory (Kelt et al., 1995): to establish themselves, our sown seeds (target species) had to pass through the mesh of the abiotic filter (since the sandy substrate formed an equivalent to a primary succession with extremely low nutrient and water holding capacity). The sown target species did not however have to overcome a biotic filter resulting from plant species already present in the habitat at the moment of their arrival. At this time point we expect that microsite limitation will have affected the germination and establishment success of the seeds the most. Our establishment data (Tables 2 and 3) back this up, with around only 30% of the sown target species managing to establish in the first growing season, and around 40% managing to establish by 2011 in both sowing treatments. Our data fit in well with results from central European grassland restoration projects (Kiehl et al. 2010) where 4 years after sowing (also at a density of 3 g/m^2) around 30–80% of target species had managed to establish, and the lower establishment rates were mainly on low-nutrient sites. This suggest that in our study microsite limitation was playing a role and affected species richness of the vegetation, even if we did not directly measure it. Kiehl et al. (2010) assessed various techniques for introducing species to a site during restoration in Central Europe and found that although sowing could overcome dispersal limitation, the long-term success of restoration also depended very much on the availability of appropriate abiotic conditions including establishment microsites.

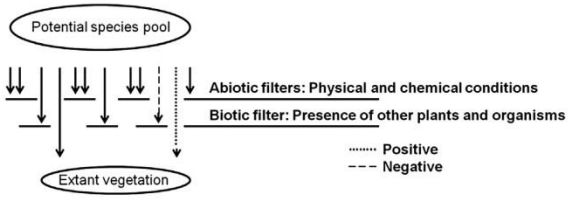

Fig. 4. Conceptual model of filter theory of community assembly based on Kelt et al., 1995 (modified from Hobbs and Norton, 2004) and adapted to include facilitation as well as competition as part of the mechanisms behind the biotic filter. The dotted arrow highlights when facilitation can help a species manage to establish, e.g. if a nurse plant provides a microsite or additional nitrogen during germination and early growth. The strongly dotted arrow indicates negative competitive interactions potentially blocking a species from managing to establish.

In classical biodiversity-ecosystem functioning experiments (where natural assembly is not allowed) more diverse communities are generally more resistant to invasion (Roscher et al, 2009). Huang et al. (2013) found in a prairie biodiversity experiment where weeding ceased after 3 years, that the positive relationship between diversity and productivity persisted even after cessation of weeding. In our study invasion pressure did increase total species number but the lack of significant difference in species number between the sowing treatments (except in year 1) suggests that invasion resistance may have been similar between treatments (Fig. 1). Overall, S7 plots had a higher establishment of target species (relative to non-target species) as well as higher soil C content and a trend to higher N content than S2 plots (Table 1). The proportion of established target species in the S2 plots significantly increased from 2008 to 2011, whereas this was not the case in S7, despite soil C remaining higher in the S7 treatment. So S7 started out with higher establishment success for target species but S2 caught up over time (Table 2). This is mirrored in the cover data where S2 started out having lower community cover than S7 but become significantly higher than S7 by 2010 and 2011 (see Fig. 1C, see 3B for target/non target species cover). This shows that the two treatments may have had similar invasion of species (in terms of numbers) but that the abundance of the species differed.

It seems that time plays a crucial role in restoration success for establishing desired target species in species-rich grasslands. Baasch et al. (2012) evaluated restoration experiments in ex-mining sites on sandy soils in eastern Germany and found that the species-rich grasslands established after hay transfer or sowing were highly resistant to invasion of ruderal species (despite hay transfer methods not only having positive effects on establishment). After 9 years, however, there was no difference between treatments in terms of total vegetation cover, species richness and the number of target species. In our study we found the same effect after only 4 years: there was no difference between sowing treatments in terms of total species number and number of target species, although productivity, community cover and functional composition did vary.

Focusing on the different functional groups of the species sown (e.g. legumes, non-legume forbs and grasses) our study found that the functional group composition sown was still detectible in the vegetation 4 years after sowing. This is an interesting effect, even if we cannot separate effects of the species richness from the functional richness of the seed mixtures.

This detectable priority effect after 4 years was valid for cover and biomass of forbs and legumes, and richness of legumes only (Table 1) but not for grasses. While one can see in our data that the higher proportion of legumes sown was reflected in higher cover and biomass of legumes over time, this effect was not found for the forbs. For the forbs, the S2 treatment that started out with less forb species, had an as high proportion of forbs in the community after 4 years as the S7 treatment. The particularly strong priority effect of sowing legumes on legume composition may be related to the finding that legumes established quickly and well. They seemed to

be well adapted to the conditions on site from the start and established well in S7, whereas in S2 the one legume species did not establish well, allowing the non-legume forbs to become dominant. We know from many greenhouse and field studies with legumes interacting with other functional groups, that legumes tend to be competitive across a range of abiotic conditions (Temperton et al., 2007; non-published data).

If in follow-up experiments the functional composition of the mixture were found to be more important than the species richness, one could perhaps use priority effects of initial sowing composition to direct the functional composition of the community as well as total aboveground biomass and cover. This is turn could potentially have positive effects on nutrient cycling and carbon sequestration in mesic grasslands (*sensu* Steinbeiss et al. 2008; Oelmann et al. 2011). This would need to be tested in separate experiments before being applicable to restoration since seed mixtures are rarely separated into these groups, but just sown as higher or lower diversity.

5. Conclusions

Overall, our study aimed to test whether priority effects of sowing differently diverse seed mixtures play a role in dry acidic grasslands, and we found that they do, but how sustained they were depended on the response variable measured. Our study found relatively low establishment success of target species, but the results are in line with results from low-nutrient grassland restoration, suggesting that microsite limitation and related filtering effects of severe abiotic environments may be the strongest driving factors in assembly of dry acidic grassland. Additional reduction of microsite limitation via planting out nurse plants to facilitate establishment in such harsh conditions or including an intermediately severe disturbance regime (as in Jentsch et al. 2009) may be as important to improving dry grassland restoration success as sowing therefore.

Acknowledgments

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6.2 Manuscript 2

Disentangling who is who during rhizosphere acidification in root interactions: combining fluorescence with optode techniques.

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Disentangling who is who during rhizosphere acidification in root interactions: combining fluorescence with optode techniques

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Plant–soil interactions can strongly influence root growth in plants. There is now increasing evidence that root–root interactions can also influence root growth, affecting architecture and root traits such as lateral root formation. Both when species grow alone or in interaction with others, root systems are in turn affected by as well as affect rhizosphere pH. Changes in soil pH have knock-on effects on nutrient availability. A limitation until recently has been the inability to assign species identity to different roots in soil. Combining the planar optode technique with fluorescent plants enables us to distinguish between plant species grown in natural soil and in parallel study pH dynamics in a non-invasive way at the same region of interest (ROI). We measured pH in the rhizosphere of maize and bean in rhizotrons in a climate chamber, with ROIs on roots in proximity to the roots of the other species as well as not-close to the other species. We found clear dynamic changes of pH over time and differences between the two species in rhizosphere acidification. Interestingly, when roots of the two species were interacting, the degree of acidification or alkalization compared to bulk soil was less strong then when roots were not growing in the vicinity of the other species. This cutting-edge approach can help provide a better understanding of plant–plant and plant–soil interactions.

Keywords: plant roots, interaction, green fluorescent protein, pH planar optodes, rhizotrons, rhizosphere, maize, bean

INTRODUCTION

The main root functions are to ensure both uptake of water and nutrient resources as well as provide an anchorage function for the whole plant. Moreover, Darwin (1880) considered roots to act as the plant brain integrating information from multiple sources. Despite these key functions of roots for whole plant performance, root ecophysiology and ecology have until relatively recently been a field of research weighed down by seemingly unsolvable difficulties in following root growth *in situ* in natural substrates. The soil–root–rhizosphere system has until recently been considered a black box that is hard to reach and to study (Faget et al., 2013).

Roots are continuously interacting with their environment, not only with their direct abiotic environment (as in the rhizosphere), but also interacting with biotic neighbors such as roots of neighboring plants, microbes, and soil fauna (Bonkowski et al., 2009). Alone when considering root interactions with the abiotic environment in the soil, processes occur at very variable spatial and temporal scales. Recent years have shown important breakthroughs in understanding the complex interplay of how roots both react to and affect their environment (de Kroon and Mommer, 2006; Schreiber et al., 2011; de Kroon et al., 2012; Postma and Lynch, 2012). It is well documented that plant roots are able to actively alter the biogeochemistry of their vicinity, the rhizosphere (Hiltner, 1904; Hinsinger et al., 2003, 2005, 2009). This interaction of plant roots with the soil causes a highly complex spatial and temporal pattern of micro niches that are potentially characterized by large differences in, e.g., soil water content, soil pH, nutrient availability, microbial community structure and activity. There are several drivers for this interaction, but root foraging for the resources water and nutrients are of most importance. Foraging and uptake of nutrients can cause strong variations in soil pH. For example, during the uptake of nitrate or ammonium, plant roots release OH^- (hydroxyl ions) or H^+ (protons) in order to maintain electro-neutrality across the root membrane (Marschner and Romheld, 1983; Colmer and Bloom, 1998; Hinsinger et al., 2003). On the other hand, plant roots are able to release large amounts of organic acids such as citric acid, in order to mobilize nutrients (e.g., phosphorous) when they are bound to soil particles and therefore inaccessible for direct uptake (Jones et al., 2003; Lambers et al., 2006). Both processes can create pH gradients of more than one pH unit from the root surface to the bulk soil. Additionally, when considering the dynamic growth of plant roots, it quickly becomes clear that the further elucidation of plant soil interactions is not a trivial task but very important for understanding plant performance, especially under stressful conditions. This point becomes even more pertinent, when the target plants are crops such as maize or bean and when the aim of the research is to sustain or even to improve the yield of crops in low-input agro-ecosystems.

When different species are sharing the same soil volume, they have to forage for the same essential resources that are often limiting and to explore and adapt to their environment to be able to uptake sufficient resources for maintaining their growth. Major

advances in root research both in ecology and ecophysiology have shown that roots respond both to nutrient availability (Hodge, 2004; Cahill et al., 2010) but also to the presence of different microfauna groups in the soil (Bonkowski et al., 2009) as well as the presence of a neighboring plant (Callaway et al., 2002; de Kroon and Mommer, 2006; de Kroon, 2007). Some studies suggest that both kin recognition (Dudley and File, 2007; Dudley et al., 2013) and recognition of the genetic identity of neighbors can influence the proliferation of roots and root allocation (Gagliano et al., 2012; Fang et al., 2013). Gagliano et al. (2012) found that the identity of the neighbor affected the allocation to roots and shoots, as well as affecting germination of seeds. Such studies finding communication between plants beyond direct resource-based competition have received a number of critical responses (Klemens, 2008), but the number of studies finding evidence for such communication is on the rise (de Kroon, 2007; Gagliano et al., 2012). This is clearly a research field with ample need for further studies to back-up and test theories and outcomes, and novel methods being established will no doubt provide important new insights to the issue of the question of plant interactions and whether non-resource-based competition is important compared to resource-based competition. Our posit, is that novel combinations of non-invasive methods for studying roots (Rewald et al., 2012; Faget et al., 2013) can now provide important tools to explore rhizosphere interactions with more ease and will allow important new insights. For further validation and elucidation of these topics an approach is missing which enables us to investigate and understand *in situ* rhizosphere processes of plants in more detail, either growing alone or intercropped with plants of different species.

Although studying the dynamics of root growth is still a challenge, new methods are allowing us to follow roots *in situ* (Faget et al., 2013) and even to separate the roots of different species (Faget et al., 2009; Rewald et al., 2012). One of these methods uses fluorescent roots of genetically transformed plant species using fluorescent protein (FP; green fluorescent protein, GFP; Faget et al., 2009, 2010, 2012, or red fluorescent protein, RFP; Faget et al., 2013). At the same time, other methods have been developed to study rhizosphere-scale processes, such as pH, CO_2, and O_2 concentrations with the technique of planar optodes (Blossfeld and Gansert, 2012; Blossfeld, 2013). The FP method relies on the ability of genetically transformed roots to express fluorescing proteins and thus be visible at certain excitation and emission wavelengths, whereas the optode method uses indicator dyes on the planar optodes that get excited by specific light and emit characteristic fluorescence patterns in proportion to the concentration of the measured substance, e.g., H^+. Planar optodes provide new opportunities to study rhizosphere processes *in situ* and dynamically over time (Blossfeld et al., 2011, 2013). There are several approaches for fluorescence detection and we refer the reader to the scientific literature for detailed comparison and evaluation of the advantages and disadvantages of the different approaches (Holst and Grunwald, 2001; Stahl et al., 2006; Gansert and Blossfeld, 2008). In studies where roots of different individuals (either of the same species or of another species) are interacting, however, it is often desirable to be able to identify which root within the region of interest (ROI) of the optode belongs to which species or genotype. For this reason, we hereby combined the GFP and planar optode methods in order to achieve the combined goal of following rhizosphere processes and being able to identify which species is which underground.

Within this context we asked:

(1) Whether we can combine the planar optode and the FP methods to visualize rhizosphere pH changes during root–root interactions between species, using the FP method to assign species identity to roots and the optode method to measure the rhizosphere pH changes.
(2) As a consequence we asked, whether we can localize specific rhizosphere processes and link them to specific plant species and their interactions?

We approached these questions by setting up an experiment with two plant species, maize and bean (*Zea mays* and *Phaseolus vulgaris*) with roots growing in rhizotrons either with or without close contact with roots of the other species. We measured selected ROIs within the rhizosphere of the rhizotrons using the planar optode method, and GFP maize to be able to identify which species is contributing to what extent to the specific pH measured in the intercropped rhizosphere.

MATERIALS AND METHODS

PLANT MATERIAL

The maize line ETH-M72$_{GFP}$ expressing the GFP was grown alone or together with common bean (*P. vulgaris* "Fadenlose"). The maize genotype ETH-M72 was genetically transformed to include the gene for GFP (ETH-M72$_{GFP}$). The transformation construct contains the gfp gene flanked by the ubiquitin promoter (ubi::gfp) and the nopaline synthase (NOS) terminator. It was cloned into the pUC19 vector, which contains the gene for ampicillin resistance (ampR) at the restriction sites *Spe*I and *Xba*I. The gfp gene was cloned into the cassette at the NcoI and SalI sites. The expressed GFP is reported to have a fluorescence peak between 500 and 520 nm when excited by light at 450–470 nm (Faget et al., 2009, 2010, 2012).

EXPERIMENTAL CONDITIONS

Seeds of the two species were germinated on blotting paper before seedlings of comparable size were transplanted into rhizotrons. The rhizotrons had one side covered in plexiglass that is removable so that planar optodes can be installed on ROIs and roots growing on the surface are visible to the naked eye. The rhizotrons with dimensions of (400 mm × 200 mm × 20 mm) were filled with 2/3 soil (sieved with 4 mm mesh) and 1/3 sand (washed two times with deionized water). The soil and sand were mixed and each rhizotron received 1.3 l of mixed substrate. All rhizotrons were kept in a climate chamber (12 h light, 240 μmol m^{-2} s^{-1} PAR, 65% humidity, 24.5°C day, and 18.5°C night). All rhizotrons were placed at an angle of 30° from the vertical with black cover on the transparent side to prevent the roots from incident light and each rhizotron received 100 ml of 1/3 the full Hoagland's nutrient solution at the start of the experiment. The rhizotrons additionally received 30 ml of 1/3 the full Hoagland nutrient solution per day. The full Hoagland nutrient solution used contained the

following minerals: 5 mM KNO_3, 5 mM $Ca(NO_3)_2$, 2 mM $MgSO_4$, 1 mM KH_2PO_4, 0.09 mM Fe EDTA, 0.01 mM $MnCl_2$, 0.001 mM $CuSO_4$, 0.001 mM $ZnSO_4$, 0.05 mM H_3BO_3, and 0.0005 mM Na_2MoO_4.

The aim of this study was to for the first time combine pH measurements using planar optodes with GFP methods in roots to discriminate between roots of different species growing adjacent to one another and hence be able to follow pH dynamics of roots whose species identity we knew.

Our setup had an intercropping factor with three levels: (i) one maize individual growing together with one bean seedling, (ii) one maize individual growing together with two bean seedlings, and (iii) a control level of one maize individual growing alone (in order to visualize rhizosphere pH dynamics without close contact between roots of the two species). The limited number of bean seedlings allowed no cultivation of single grown bean plants, therefore we used ROIs of bean roots growing without neighbors within the intercropping rhizotrons. So, for example, in **Figure 3**, the pH data depict values for maize from maize growing in its own rhizotron, whereas the pH data for bean depict values from a bean root growing without close neighbors but in an intercropped rhizotron. In intercropped rhizotrons we therefore had two ROIs with planar optodes attached, corresponding to maize root next to bean, bean root growing without a neighbor.

It is important to note that the spatial scale of a planar optode ROI is very much smaller than that of the whole rhizotron, such that we considered the ROIs as replicates in most cases [e.g., see **Figure 5**; see arguments in Hurlbert (1984) on the issue of spatial scale and pseudo-replication in experiments]. Seedlings were transplanted into four rhizotrons per factor level (i.e., $n = 4$) on Day 1.

Four days after transplanting (DAT), all roots had reached half way to the bottom of the rhizotrons. Planar optodes were placed into the rhizotrons on 5 DAT. The number of optodes was limited and therefore not all rhizotron replicates could be investigated at each time point: for the evaluation of pH dynamics particular ROIs within every single optode where determined according to the following scheme: central on surface of maize/bean root, bulk soil close to maize/bean root (i.e., 6–10 mm off the root surface) and bulk soil between the roots of both species.

Additionally, it turned out that after the placement of the optodes two intercropping rhizotrons could not be included in the further analysis because the roots of maize and bean grew together too close in order to separate individual pH signals.

During the course of the experiment, some ROIs showed an unexpectedly strong pH drop which was out of the range of the calibration curve (see chapter below). This caused a reduction of number of replicates during data analysis. In particular the number of replicates changed as follows: maize $n = 4$ DAT 6–8 and 14, $n = 3$ DAT 12; bean $n = 4$ DAT 6–8, $n = 3$ DAT 12–14; bulk soil close to bean/maize $n = 4$ DAT 6–14; bulk soil between roots $n = 4$ DAT 6–8, $n = 2$ DAT 12–14. Conventional and fluorescent pictures (for FP) as well as pH measurements using the planar optodes were taken on the following days of the experiment: 6 (morning and afternoon), 7 (afternoon), 8 (afternoon), 12 (afternoon), and 14 (morning).

GFP TECHNIQUE

To identify the plant species crossing the optode region in a first step, the plant roots of maize and bean grown along the transparent plate of the rhizotrons were imaged with a conventional camera system and with an adapted lighting system-filtered camera to excite the FPs as described in Faget et al. (2009). In this paper, to adapt the previously developed method for minirhizotron to rhizotron with the transparent plate, we used a digital camera Canon, G10 mounted on a tripod. The conventional camera systems use ambient light and photograph the roots at the interface of the soil with the transparent plexiglass window of the rhizotrons. For the adaptation of this system to GFP, we mounted a filter (LONG 515 nm, Edmund Optics, Barrington, USA) in front of the camera to allow only roots expressing the GFP to be visible under excitation light (at wavelengths of 440–460 nm); further details including the components and standardized protocol are given in Faget et al. (2010).

At harvest, a closer identification was necessary to assess the root identity under the optode by removing the sensor and re-screening this area with conventional and fluorescent imaging techniques.

OPTODE TECHNIQUE

Depending on the optical setup, different spatial and temporal resolutions can be achieved. In our experimental design we used the setup as recently described in Blossfeld et al. (2013). In particular, we used a fluorescent detection system with a field of view of 15 mm × 12 mm and a pixel resolution of 12 µm. In detail, this detection system is based on a modified USB-microscope device that consists of a light-emitting diode (LED) ring (470 nm) functioning as the excitation light source, filters, lens, and the complementary metal-oxide-semiconductor (CMOS) chip. The detection system is connected via USB to a PC and powered by this connection. Thus, this system is highly flexible and even portable, when using a notebook. The RGB images [24-bit, 1280 × 1024 (1.3 megapixel)] created by this detection system contain the raw, i.e., untreated sensor response. Hence, these red, green, blue (RGB) images needed to be analyzed with an image processing software (VisiSens; PreSens GmbH, Regensburg, Germany). This software calculates the ratio of red to green in the emitted fluorescence response (so-called *R*-value) provided by the color channels of the CMOS chip. This is possible because the optodes were made of two different dyes that are either analyte-sensitive or analyte-insensitive. The intensity of the green fluorescence of the analyte-sensitive dye is driven by the analyte concentration, whereas the intensity of the red fluorescence of the analyte-insensitive dye is not. The CMOS chip captured the red and green fluorescence in one single image and therefore the created *R*-value then provided a two-dimensional quantitative map of the measured parameter, i.e., the pH.

Several optode sensor foils (size 10 mm × 20 mm, product code SF-HP5-OIW; PreSens GmbH) were fixed at the transparent front plate of the rhizotrons with plants growing in them. The positioning was done 5 DAT when the roots had reached almost the lower third of the rhizotrons. By adding the planar optodes at this time point, we ensured that the placement of the planar optode was at a ROI (size 2 cm × 1 cm). We chose our ROIs in the

following manner: we placed the optode on a zone where the tip of a growing root was just inside the area covered by the optode; this allowed for measurement of pH changes in most of the optode region without direct root contact at time point zero, as well as the dynamic measurement of pH changes as the root(s) grew through the ROI, i.e., behind the optode.

The rhizotrons were closed again after the placement of the planar optodes and first daily measurements were performed after one day of equilibration. The soil moisture and temperature was monitored in four rhizotrons via frequency domain reflectometry (FDR)-probes (Model: 5TE, Decagon Devices Inc., 2365 NE Hokins Court, Pullman WA 99163) parallel to the daily measurements and ranged between 26.6 and 36.3% (volumetric water content, VWC) as well as 24.3 and 25.1°C in the afternoon.

CALIBRATION OF PLANAR OPTODES

Prior to the start of the experiment, the optical setup together with the planar optodes was calibrated. This was achieved by using a small transparent vessel containing defined pH buffer solutions (mixture of K_2HPO_4 and KH_2PO_4, controlled with standard pH glass electrodes) and a small replicate of the planar optode batch installed on the inside of this vessel.

The average R-value (R_m) of this replicate for each given pH was recorded and used as input parameter for a fitting function. The relationship between R_m and the given pH can be described by a sigmoidal Boltzmann equation (Eq. 1). This equation was adapted from (Blossfeld and Gansert, 2007) by exchanging the parameter Φ with the parameter R. This equation can be transformed in order to calculate the pH from the measured R-value during the experiment (Eq. 2). This equation was also adapted from Blossfeld and Gansert (2007) by exchanging the parameter Φ with the parameter R.

$$R_m = \frac{R_{min} - R_{max}}{\{1 + \mathrm{Exp}\left[(pH_m - pH_0)\, dpH\right]\}} + R_{max}, \quad (1)$$

$$pH_m = pH_0 + dpH \times \ln\left[\frac{(R_{min} - R_{max})}{(R_m - R_{max}) - 1}\right], \quad (2)$$

where R_m is the calculated/measured R-value, R_{min} and R_{max} represent the upper and lower range of the fitting; pH_0 is the inflection point and dpH the slope of the fitted curve. The Boltzmann fit clearly demonstrates that the sensitivity of the sensor was highest between pH 6 and pH 7 and lowest below pH 5 and above pH 8 (**Figure 1**).

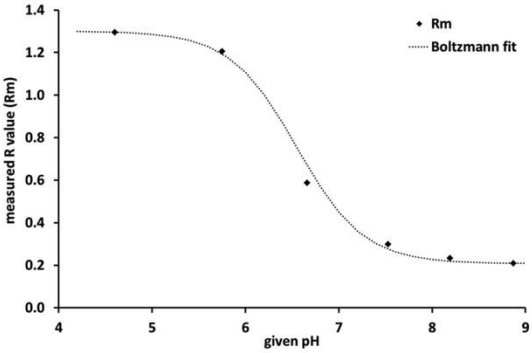

FIGURE 1 | Calibration curve of the planar optodes, where R_m is the measured R-value, i. e., the ratio of red to green in the emitted fluorescence response. The steep slope of the Boltzmann fit between pH 6 and pH 7 indicates that the sensor is most sensitive within this pH range.

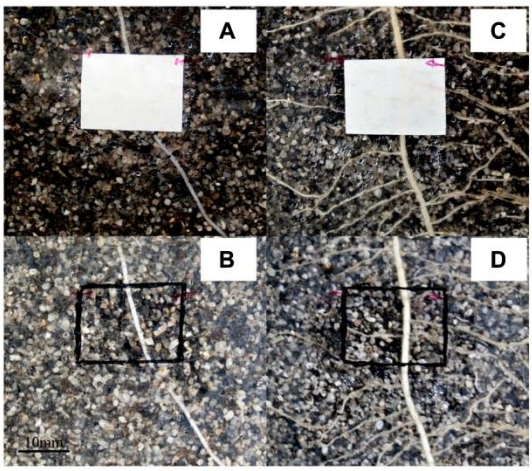

FIGURE 2 | Photographs of the experimental setup as seen through the transparent window of the rhizotrons with and without optodes installed. The pictures show roots growing with no neighbor of another species nearby. Panels **(A,B)** are photographs of maize roots while **(C,D)** are of bean roots. Panels **(A,C)** were shot at the time of destructive harvesting which corresponded to DAT 14 (DAT, days after transplanting) and we can clearly see the roots crossing the planar optodes. The optodes where removed as seen in **(B,D)** to precisely locate the root trajectories under the sensors. The scale is given by the optodes which measure 10 mm × 20 mm regions of interest (ROIs).

RESULTS AND DISCUSSION

Figure 2 shows photographs through the transparent window of the rhizotrons of maize roots on the left side (**Figures 2A,B**) and bean roots on the right side (**Figures 2C,D**) growing alone with no neighbors in the proximal rhizosphere. The upper row (**Figures 2A,C**) was taken before harvest showing the position of the planar optodes on the root systems through the window interface. Just before harvest, the planar optodes were taken away to identify and measure the exact location of the roots behind the optode sensors (**Figures 2B,D**).

The pH monitoring via the optodes revealed that the investigated species modified their rhizosphere pH creating very distinctive patterns; **Figure 3** shows the evolution of pH measured by the planar optodes over time. We found clear dynamic changes of pH over time and differences between the two species in rhizosphere acidification both when roots grew alone and in interaction between the species.

Initially, the roots of maize growing alone acidified the rhizosphere on average by 0.75 pH units compared to the bulk soil pH (**Figure 3A**). This rhizosphere acidification was not constant over time, but changed instead to a net alkalization of up to 0.62 pH

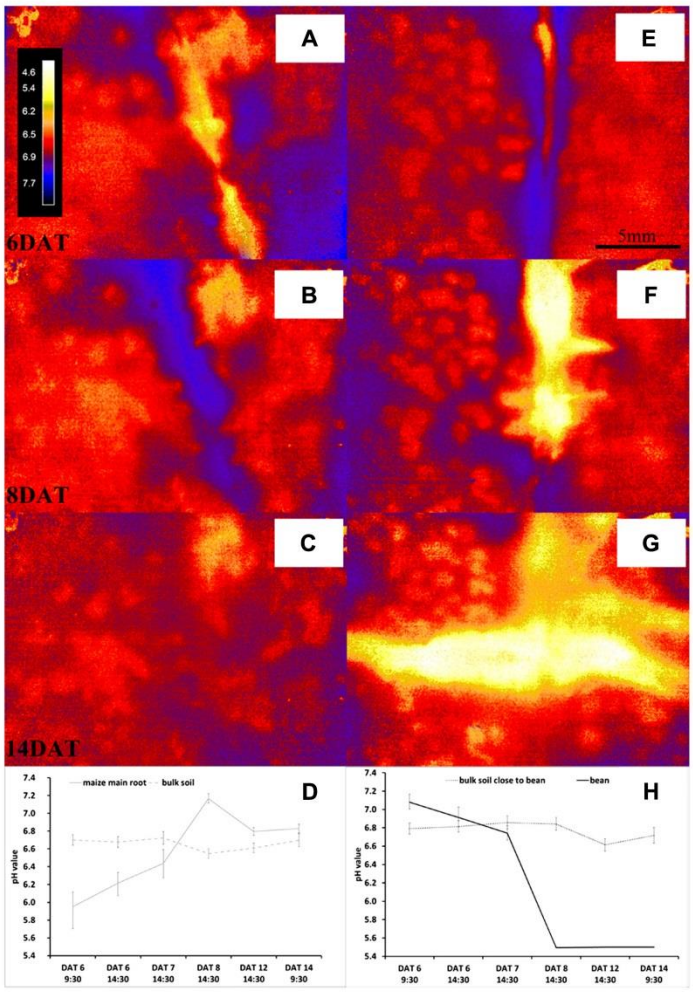

FIGURE 3 | Dynamics over time of pH measured with the optodes for the rhizosphere and bulk soil of roots of maize (A–D) or bean (E–H) growing alone. Panels **(A–C,E–G)** show the pH maps of the respective ROIs at a scale ranging from 4.6 to 7.7 pH units at DAT 6 **(A,E)**, DAT 8 **(B,F)**, and DAT 14 **(C,G)**, respectively; the scale here is 20 mm × 10 mm. Panels **(D,H)** show the evolution of the mean pH value (±SD) of all pixel within the ROI at the root surface of maize **(D)** and bean **(H)** over time growing in separate rhizotrons.

units on 8 DAT (**Figure 3B**). In the later phase of the experiment the rhizosphere pH came closer to the bulk soil pH, which varied between pH 6.55 and pH 6.72 (**Figure 3C**).

Interestingly, the single grown bean roots showed the opposite behavior (**Figures 3D,H**). The rhizosphere of this young bean roots was 0.29 pH units higher than the initial bulk soil pH of 6.79 (**Figure 3E**). However, from 8 DAT onward, the bean roots acidified the rhizosphere in such a strong manner that the sensor signal was below pH 5.5 (**Figure 3F**). The young lateral roots of bean acidified the rhizosphere right from their emergence onward and it cannot be excluded that some of the acidic molecules diffused along the lateral roots to the main roots (**Figure 3G**). It should also be noted that the bean roots formed no nodules during the course of the experiment. Since both species were grown in the same substrate and all rhizotrons received the same watering regime with the same nutrient solution, this contrasting pattern is very interesting. Since the only source of nitrogen in the rhizosphere of all plants was derived from the nitrate of the nutrient solution, we expected that an uptake of this nitrate would cause an alkalization of the rhizosphere (Marschner and Romheld, 1983; Colmer and Bloom, 1998; Cousins and Bloom, 2003). This was what we found around the maize roots, but not the bean roots, although we cannot confirm with our study that this is the mechanism behind the pH patterns we found. The bean rhizosphere pH response is

difficult to interpret given that there were no nodules on the roots and hence no sign of N_2-fixation occurring, which would have potentially explained the acidification over time as protons are released during fixation (Bolan et al., 1991). Another explanation could be the species-specific ability to mobilize phosphorous (P) in the rhizosphere. It has been reported that under P-limitation but high nitrate content non-nodulated roots of faba bean heavily acidified the rhizosphere, whereas maize roots alkalinized their rhizosphere when growing under the same conditions (Li et al., 2007). However, we have not measured the P-content of the plants and the soil after the experiment in order to verify this explanation. Thus, the patterns found now need further testing with more replication, further soil, and plant analysis and with a variety of species in addition to maize and bean.

Figure 4 shows the evolution of pH over time when roots of both species grew within the proximity of the other. **Figures 4A–C** show the variations in acidification and alkalization of the rhizosphere at 6, 8, and 14 DAT, respectively. **Figures 4D,E** allow us to identify which roots belonged to which species (using the GFP method and conventional photography): in interaction, we see an acidification, then alkalization followed by an acidification of the maize root, with a clear acidification of the bean root over time (**Figures 4A–C**). Overall (see **Figure 5** for more detailed views of the pH changes) we found that the pattern of rhizosphere acidification over time was similar to that found when the roots of one species were not in the proximity to the other, but the intensity of the pH changes was about 0.6 pH units lower.

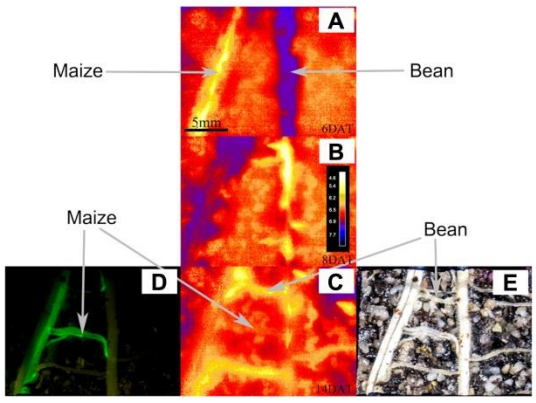

FIGURE 4 | This figure shows the potential of combining fluorescence (GFP) with optode pH methods, illustrating what each method can contribute to understanding who is who during rhizosphere pH changes. The figure shows the ROIs for the rhizosphere and the bulk soil of roots of maize and bean growing in close proximity to each other. Panels **(A–C)** show the pH maps of pH measured with the optodes for the rhizosphere and bulk soil of roots of maize and bean growing in close proximity: at a scale ranging from 4.6 to 7.7 pH units at DAT 6 **(A)**, DAT 8 **(B)**, and DAT 14 at the end of the experiment **(C)**. Panels **(D,E)** show photographs of these same ROIs taken on DAT 14 at harvesting after having removed the optode for locating and identifying roots. Panel **(D)** was photographed under blue light to excite the maize expressing the GFP, allowing the identification and exact location of the maize roots (the roots tips and meristematic areas are even brighter than the remaining tissue). Panel **(E)** shows a conventional photograph that is complementary to **(D)** shot in conventional light, where all the roots form maize and bean are visible.

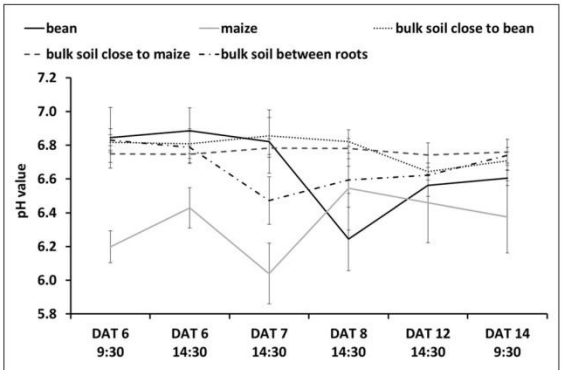

FIGURE 5 | pH Dynamics over time as related to different ROIs either in the rhizosphere of maize or bean (i. e., positioned centrally on the individual root), in the bulk soil close to maize or bean roots (i.e., positioned 6–10 mm away from the individual root), or in the bulk soil between maize and bean roots (i.e., positioned centrally between roots with >6 mm distance between them). Values are means and standard errors of the mean of all pixels (approx. 2500–3500 pixels for the rhizosphere and 10000–15000 pixels for bulk soil) in the individual ROI. Note that these mean values are derived solely from intercropping rhizotrons, such that they are composed of values from rhizotrons with maize intercropped with one or with two bean individuals. For maize $n = 4$ DAT 6–8 and 14, $n = 3$ DAT 12; bean $n = 4$ DAT 6–8, $n = 3$ DAT 12–14; bulk soil close to bean/maize $n = 4$ DAT 6–14; bulk soil between roots $n = 4$ DAT 6–8, $n = 2$ DAT 12–14.

We cannot yet explain why we found a less strong change in pH compared to bulk soil (**Figure 5**) when roots of the two species were directly interacting. Further studies should help identify whether this was due to the species interactions and some kind of plant–plant communication or other more resource-based competitive outcomes (see Faget et al., 2013 for discussion of this topic).

Without the GFP method it would have been impossible to distinguish by eye, which root belonged to which species and thus which pH activity could be assigned to the maize or to the bean root zones (as is the case in **Figures 2** and **3**). At harvest time (14 DAT) the optode was removed and the roots were imaged (**Figure 4E**). This conventional photograph is helpful to visualize the location of different roots behind the optodes but alone does not allow one to identify to which species they belong. By using the GFP method, as in this case maize roots expressing the GFP, it was possible to separate maize from bean roots and to then compare pH dynamics in specific ROIs.

Figure 4D clearly shows the maize roots in fluorescent green, differing from the bean roots in pale color or even not visible on the GFP-image but only on the conventional image. Here we can see that some of the lateral roots belonged to maize and some to bean, which would not have been visible to the naked eye. This then explains why not all visible lateral roots acidified their rhizosphere and why the acidification of the upper and lower lateral roots is not as prominent as in the single root observations (**Figure 5**).

In **Figure 4D** one can clearly see that only the acidifying roots belong to the bean plant and the central lateral roots belong to the

maize plant. Hence, by combining the GFP method with the planar optode methods, it is now possible to follow the pH variation of the rhizosphere during plant–plant interactions and precisely indicate which species had what kind of influence on the rhizosphere properties, even if the mechanism behind the patterns requires further complementary studies.

Combining these methods should also allow one to compare the integrated effect of roots growing alone or with neighbors on rhizosphere pH (or O_2 or CO_2) with outcomes when roots are interacting directly. For example in our study, we found that the modification of the rhizosphere pH when roots of two species were directly interacting was similar to the roots growing alone (**Figure 5**). The maize still tended to alkalinize the rhizosphere and the bean still acidified it, but the intensity of the modification by the roots of both species was reduced.

We also found that the pH of the bulk soil in proximity of either the maize roots or bean roots did not show strong variation while the pH of the bulk soil in between the two roots systems suggests it may be an averaging of the pH values for roots growing alone.

Our approach has the potential to prove very useful in so-called *guided sampling*. High-throughput phenotyping of plant traits is currently a burgeoning field in plant sciences (Rascher et al., 2011; Nagel et al., 2012; Fiorani and Schurr, 2013), and allows for large screening of many genotypes and species. At times, high-throughput phenotyping can benefit greatly from more detailed lower-throughput methods such as the described planar optode method for studying processes in the rhizosphere at particular points in space or time deemed particularly interesting. The planar optode method can report differences in rhizosphere (metabolic) activity of different roots, including hotspots of root activity in the main or lateral roots at different times. Information derived from the optodes and the GFP-images could then be used directly for guided sampling of specific root/rhizosphere sections for analysis of compounds, enzymes, microbial communities, etc. One of the main limitations would come from the need to use genetic modified plant material. This is a pre-condition in order to be able to distinguish roots from different species. GFP-transformed *Arabidopsis thaliana* is readily available, whereas it is not available yet for many other plant species since transformation involves a considerable amount of work.

Another area of research where we deem that the application of these two methods may be very promising is in plant–plant interaction studies in ecology and ecophysiology. In these research fields, a range of different theories to explain patterns found in nature are being tested based on both resource-based and non-resource-based competition, novel communication pathways between plants (Zavala and Baldwin, 2006; Gagliano et al., 2012), as well as considering the role of positive interactions between plants as well as competition (Temperton et al., 2007; Brooker et al., 2008).

Not only GFP is available but different colors have now been made available in a number of mainly agriculturally interesting species which will make possible for us to be able to distinguish and study root–root interactions within populations as well as communities in the longer run. For example, maize expressing the GFP was combined with wheat expressing the RFP and rapeseed as wild type in Faget et al. (2013). At the same time, planar optodes can measure not only pH but also CO_2, O_2, and ammonium (Stromberg, 2008) and the size of the optodes available for research is increasing such that whole rhizotrons can soon follow plant–soil dynamics over time. This combination of novel methods for studying root biology and ecology should pave the way to an improved understanding of both root–soil and root–root interactions.

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6.3 Manuscript 3

Priority Effects of Time of Arrival of Plant Functional Groups Override Sowing Interval or Density Effects: A Grassland Experiment.

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Priority Effects of Time of Arrival of Plant Functional Groups Override Sowing Interval or Density Effects: A Grassland Experiment

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Abstract

Priority effects occur when species that arrive first in a habitat significantly affect the establishment, growth, or reproduction of species arriving later and thus affect functioning of communities. However, we know little about how the timing of arrival of functionally different species may alter structure and function during assembly. Even less is known about how plant density might interact with initial assembly. In a greenhouse experiment legumes, grasses or forbs were sown a number of weeks before the other two plant functional types were sown (PFT) in combination with a sowing density treatment. Legumes, grasses or non-legume forbs were sown first at three different density levels followed by sowing of the remaining PFTs after three or six-weeks. We found that the order of arrival of different plant functional types had a much stronger influence on aboveground productivity than sowing density or interval between the sowing events. The sowing of legumes before the other PFTs produced the highest aboveground biomass. The larger sowing interval led to higher asymmetric competition, with highest dominance of the PFT sown first. It seems that legumes were better able to get a head-start and be productive before the later groups arrived, but that their traits allowed for better subsequent establishment of non-legume PFTs. Our study indicates that the manipulation of the order of arrival can create priority effects which favour functional groups of plants differently and thus induce different assembly routes and affect community composition and functioning.

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Introduction

Research into the assembly of ecological communities has shown that the extant composition of communities is strongly influenced by historical factors [1–3]. Priority effects occur in communities, when one (or more) species already is present in a habitat and thereby affects the success of later species [4,5], and this effect can be either negative, positive or neutral [6]. The success of other species can relate to their establishment, growth or reproduction [7]. Priority effects are thus important e.g. to understand when applying ecological theory and knowledge to help restore degraded habitats where certain species are introduced to a site via restoration (Grman and Suding 2010). Species arriving prior to other species are generally considered to either affect newcomers via size-asymmetric competition [8] or so-called legacies in the soil created by effects of plant-soil feedback on the soil [9]. Another possible mechanism of priority effects is nitrogen (N) facilitation (including N transfer and N sparing) between N_2-fixing species arriving early during assembly and other functionally different species arriving at a later time-point (see Körner et al. [10] for first indication of this). No matter the mechanism, the outcome of priority effects seems to be that competitive and or facilitative interactions for newcomers are altered.

Priority effects can lead to lasting differences in species or functional composition, and hence can potentially drive ecosystem properties and functioning, and may sometimes even have a stronger influence than the effects of abiotic conditions on community composition [1,11]. In aquatic model-ecosystems also, there is evidence that properties, such as biomass production or community size, seem to be more dependent on initial arrival order and frequency than on other factors such as initial species richness [4,12]. Recent research has found a mediating role of soil resource availability in relation to the importance of priority effects, however, at least in a pot experiment [13].

Recent research has focused on two different kinds of priority effects in plant assembly, the one showing long-term effects on vegetation caused by adding species mixtures at the same time [14,15] or altering the sequence of arrival of different species or groups of species [10,11,13]. Although the simultaneous introduction of species is of high relevance to restoration projects where mixtures of plants are often used, the potential mechanisms of order of arrival of in particular different functional groups has not been much explored yet. N_2-fixing legumes are known to be

ecosystem engineers, in particular introducing extra N_2 into soils and hence driving N cycling and community productivity [16]. We now know from many biodiversity experiments that niche complementarity between species varying in traits can lead to better overall resource-use at community level, and that particular combinations of functional groups (particularly N_2-fixers combined with grasses) as well as species richness can drive positive diversity effects [17–20]. It may be that this complementarity between different functional groups is a driver of assembly over time, and hence composition and functioning of communities.

Körner et al. (2008) varied the arrival order of three different plant functional types (from hereon called PFTs) each containing two out of six plant species in microcosms, with either legumes, non-legume forbs, or grasses sown first and the other two groups sown three weeks later. They found strong priority effects of sowing legumes first on both aboveground and belowground community productivity, even after two growing seasons. In their study the set of species in each microcosm was comparably small in relation to the biodiversity of common grasslands in central Europe. To be able to set the outcome of such a study into a more applied context (e.g. restoration or creation of semi-natural grasslands) it is essential to look on the species which occur naturally in such environments. In particular to enhance restoration of species-rich grasslands, the role of legumes as possible ecosystem engineers on nutrient-poor soils needs further research.

As the number of species in a system increases so does the number of possible interactions, either positive or negative thus affecting assembly [21–23]. We know from many biodiversity experiments that niche complementarity between species varying in traits can lead to better overall resource-use at community level, and that particular combinations of functional groups (particularly N_2-fixers combined with grasses) as well as species- and functional group richness can drive positive diversity effects [17–20].

Species that arrive first at a site have a competitive advantage over those that arrive later, and the longer the time interval between establishment episodes the more asymmetric competition may become [8]. The relative benefit one PFT can get through this competitive advantage of arriving first, however, might become a benefit for the whole community when these species have special traits such as legumes due to their ability to increase N availability either via N sparing or via N transfer. Therefore especially in harsh environments (e.g. low initial nutrient content or high environmental stress) legumes may have a positive effect not only on productivity but also on other species survival and establishment and thus positively influencing assembly [24]. Positive effects found on productivity by sowing legumes before other functional groups [10] were related to a three-week sowing interval. To what extent the sowing interval affects assembly outcomes now needs further study, since the ontological state (life stage) of a plant population may influence the species interactions and hence priority effects.

Community assembly in general and priority effects are in all likelihood modulated by both density of individuals in communities as well as environmental resource availability [8]. The law of constant yield predicts that even-aged populations grown in different densities show the same overall productivity after a certain period of time [25]. Where initial biomass is higher with increasing density this relationship wears off with time leading to the same productivity of standing biomass independent of the population density (with higher individual numbers in high densities but lower standing biomass per individual). Competition for nutrients is considered the key mechanism behind the constant yield law, but size-density relationships may change in different environments [26]. Under more extreme environmental conditions, for example, facilitation may drive communities as much as competition does. The size of an individual does not necessarily decrease with increasing density. Indeed, if facilitation and competition take place simultaneously, the size of individuals may even increase with density. In addition, sowing at high densities is often associated with higher cover values and relative abundance of sown species [27,28] correlating with greater productivity. In this sense increasing sowing density could potentially have a positive effect on productivity. However size-density-yield relationships especially in mixed stands have rarely been investigated.

This study investigated the effect of order of arrival (priority effect) of functionally different species groups (PFTs) on the productivity as well as species and functional composition of species-rich grassland communities grown in pots under greenhouse conditions. The experiment was multi-factorial regarding order of arrival, density and sowing interval as factors tested for their effects on community productivity and composition. The following hypotheses were tested:

1) The longer the sowing interval between the PFT sown first and the subsequently sown PFTs the lower the aboveground productivity of the system will be. This is due to stronger asymmetric competition between PFTs when early arrivers get a head-start and very little complementarity between PFTs can occur.
2) Sowing different seed densities will result in higher individual numbers at higher sowing densities but overall aboveground productivity will remain the same across all levels of the density treatment due to the law of constant yield.

Materials and Methods

Experimental Setup and Initial Conditions

A pot experiment was set up in the greenhouse of the Institute of Bio- and Geosciences (IBG-2), Germany in April 2011 sowing seeds typical of mesic and dry grassland habitats in the region. The experiment lasted from May until August (a total of 18 weeks from first sowing to harvest). A total of 28 typical central European grassland species were selected belonging to the three plant functional types forbs, grasses and legumes (PFT: 14 forb-, 7 grass-, 7 legume species; for species list see Supporting Information, Table. S1: Plant species per functional group with respective seed mass per pot). We chose this relative contribution of the three PFTs based on relative abundances in natural or semi-natural communities in such grasslands in Germany, (Matthias Solle, personal communication) known to have different effects on nutrient cycling and productivity from biodiversity experiments [18,29]. Species selection was based on broad phytosociological units of the given grassland communities in dry to mesic conditions [30] and Ellenberg's indicator values [31].

Experimental Design

Pots with a volume of 5 litre and an upper diameter of 20 cm and a diameter of 15 cm at the bottom were filled with a 1:2 mixture of sand (grain size 0.7–1.4 mm) and low nutrient potting soil (Einheitserde- und Humuswerke GmbH & Co. KG; "Typ P") as a substrate (for initial nutrient status of the soil see Supporting Information, Table. S2: Results of soil analysis at the beginning of the experiment). By using a substrate with low initial nutrient status we wanted to foster effects of positive and negative plant-

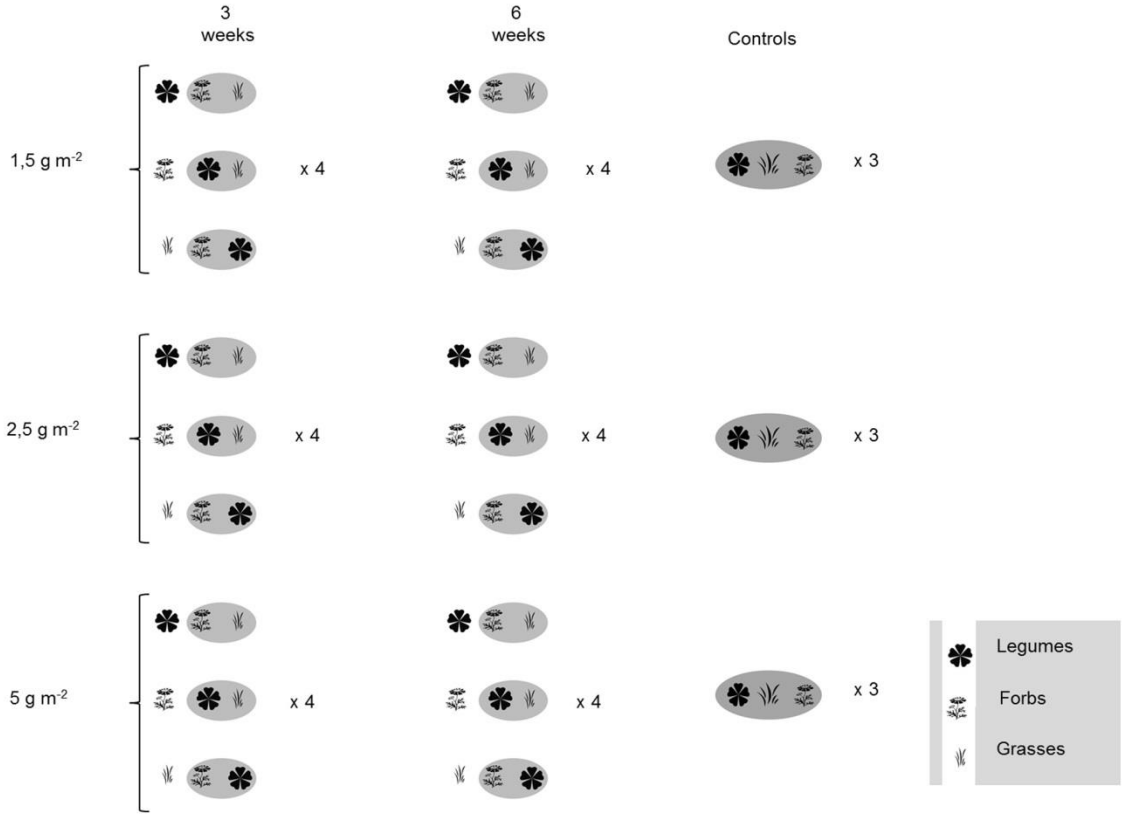

Figure 1. Experimental setup showing the three different treatments of the experiment: the priority effect treatment of arrival order, the different density treatment and the sowing interval treatment. Priority effects of early sowing of one PFT are shown with a plant functional group symbol without a circle, and later sowing of the remaining two PFTs are shown in grey circles. For the priority effect treatment legumes, forbs or grasses were sown a number of weeks before the other two groups. Density levels were 1.5, 2.5 and 5 g m^{-2}, and sowing intervals were three- and six-weeks between first PFT sown and remaining PFTs. Controls involved all PFTs being sown together at the same time. Number of replicates is shown in bold next to each treatment.
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plant interaction to be reflected in productivity and species composition. Sand was added to increase water permeability.

Plant species density was standardised on behalf of their seed mass (giving a sowing density) and records of germination capacity. Seeds were obtained from Rieger-Hofmann GmbH and mixed manually to form a density treatment with three different levels (1.5; 2.5 & 5 g m^{-2}). Before sowing, densities were calculated on the basis of the thousand-seed weight (*TSW*) of each of the species (for species list see Supporting Information, Table. S1: Plant species per functional group with respective seed mass per pot) and an empirical value derived from germination tests (*A*) standing for number of individuals of species "x" m^{-2} (Matthias Stolle, Rieger-Hofmann GmbH, personal communication) for pot surface area (*B*) and a factor (*Y*) to meet the desired plant density level, as follows:

$$((A * TSW(g))/1000) * Y) * B$$

Assembly order was influenced through a variation in order of arrival (sowing time) of three different PFTs. Species groups referred to as PFTs were non-legume forbs (hereafter referred to as forbs), nitrogen-fixing Fabaceae (legumes) and grasses. Four priority effect treatments (PE) were set up: forbs sown first (F-first), grasses sown first (G-first), legumes sown first (L-first) and a control treatment with all PFTs sown together at the same time. The priority effect treatment was created by sowing one PFT first on one sowing date (13-April-2011) and the other two remaining PFTs respectively three-(04-May-2011) or six weeks (25-May-2011) later providing a sowing interval treatment of either three- or six weeks. Each priority effect- and sowing interval treatment was additionally sown at three different density levels giving four replicates per PE-, density- and sowing interval-treatment (Fig. 1).

Pots were watered continuously by an automated irrigation system (Gardena) using rain water. Water was allowed to drain from the pots through holes in the bottom. Temperatures in the greenhouse varied from 17°C at night to 25°C in the day during the experimental period. Sowing occurred in all 81 pots one week after the filling of to allow the substrate to rest. Three soil samples were taken at time zero to evaluate the nutrient status at the beginning of the experiment (Supporting Information, Table. S2: Results of soil analysis at the beginning of the experiment). Pot

Table 1. ANOVA table for the effects of experimental treatments on aboveground biomass.

Treatment	S.S.	d.f.	M.S.	F	p	Partial-η^2
PE	1.667	3	.556	82.527	.000	.813
Sowing_Interval	.399	1	.399	59.313	.000	.510
Density	.075	2	.037	5.567	.006	.163
PE * Sowing_Interval	.151	3	.050	7.466	.000	.282
PE * Density	.040	6	.007	.983	.445	.094
Sowing_Interval * Density	.004	2	.002	.307	.737	.011
PE * Sowing_ Interval * Density	.038	6	.006	.937	.476	.090
Error	.384	57	.007			

ANOVA table for effects of the experimental treatments (arrival order (PE), sowing density (density) and sowing interval) and their interactions on aboveground biomass production. Effect sizes are calculated as partial η^2.
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distribution followed a randomised design and pot positions were changed at one time point during the experiment to take account of microclimate effects. In the case of colonisation by non-target species, pots were weeded (four times during experiment).

The response variables measured were: aboveground biomass, cover and number of individuals per plant species.

To identify treatment effects on plant community composition we assessed plant cover per species at one time point during the experiment at the time point of peak biomass development, 81 days after the first initial sowing. These estimates were performed using a modified cover estimation method following Braun Blanquet and further modified by Londo [32]. In addition to estimated cover per plant species, numbers of individuals per species were counted in each pot.

At the end of the experiment, total aboveground biomass was determined through a destructive harvest (for each of the two sowing intervals it was 78 days after the second sowing). Although the start of both sowing interval groups was at the same time, the end of the experiment was at two different time points depending on the sowing interval treatment (21-Jul-2011 & 12-Aug-2011). The different harvesting dates for these two groups (three- or six-week interval) were chosen to allow the latter sown remaining PFTs to have the same time to develop in both sowing interval treatment groups. At harvest aboveground plant parts were cut 2 mm above the soil surface, separated into PFTs, and oven-dried at 70°C to constant weight. For the first harvesting date (21-Jul-2011) only one of the three control replicates was harvested, leaving the remaining two for the second harvesting date. In addition, soil samples were taken from each pot to evaluate the nutrient status for nitrate, nitrite, ammonia, phosphorus and potassium. Measurements were performed after extraction with an 1 M KCl solution and following measurement in a Dionex ICS-3000 (except for potassium which was analysed in an 0.1 M CaCl solution with an ICP-OES). Total carbon and nitrogen in the soil were measured using an element analyser (VarioelCube, Elementar).

Statistical Analysis

The experiment was multi-factorial in design with three main factors: priority effect of arrival order, sowing interval and density. The priority effect factor had four different levels (F-first, G-first, L-first and control sown at the same time). The sowing interval factor had two levels (three- and six weeks between early sowing of first PFT and subsequent sowing of the other two PFTs). The density factor had three levels (1.5, 2.5 and 5 g m^{-2} seeds sown). Response variables included aboveground biomass at community level and at population level plant cover per species, to assess species composition but also community structure.

Number of plant individuals per pot were analysed using a one-way ANOVA testing for the effects of density and sowing interval independently. Treatment levels were tested against each other by performing Tukey contrasts. This method enabled us not only to test for general treatment effects but to test each single level of a treatment specifically in relation to each other without increasing the chance of a type one statistical error.

Communities' similarities were depicted by a dendrogram resulting from a hierarchical cluster analysis on the basis of a distance matrix (between group linkages). Distances were calculated on behalf of individual species' occurrence and cover by using Pearsons' correlation coefficient.

Biomass data was analysed using three-way ANOVA testing for effects of the factors PE, sowing interval and density as well as any interaction effects between these factors (for ANOVA Table see Table. 1). The experimental design was almost balanced and orthogonal for the three factors, except that for the three controls replicates (i.e. all PFTs sown at same time), one replicate was harvested at the first harvesting date and the remaining two at the second harvesting date. Data was generally analysed using Type III ANOVA but also using Type I ANOVA. Type I ANOVA allows to alter the order and thereby take into account the relative variability explained by this factor (see Oelmann et al. [33]) depending on when it is fitted in the model. Type I allowed us to therefore test relative effects of the three factors, depending on when they were fitted in the model.

Normal distribution of the residuals and homogeneity of variance were checked with pp-plots and Levene's tests respectively. Any data that did not fulfil the assumption of homogeneity of variance and normal distribution of the residuals were transformed (log 10) before analysis. Effect sizes for each factor as the proportion of explained variance were calculated as partial η^2. Analyses were run using PASW Statistics 18 (formally known as SPSS; IBM).

Results

Priority Effect of PFTs on Aboveground Productivity

The early sowing of one PFT (PE treatment) had a significant effect on aboveground plant productivity (Fig.2; $F_{(3,\ 57)} = 82.527$, $P<0.0001$).

Within the levels of the priority effect treatment, communities in which legumes were sown first (L-first) were the most productive (especially when sown at high density) with aboveground biomass ranging from 664±92 g m^{-2} to 1608±126 g m^{-2} followed by G-first (ranging from 521±37 g m^{-2} to 751±19 g m^{-2}) and F-first (ranging from 389±20 g m^{-2} to 570±44 g m^{-2}). The L-first treatment with the densities 1.5 g m^{-2}, 2.5 g m^{-2} and 5 g m^{-2} being on average 25.3%, 30.5%, 27.8% more productive than their respective controls in the six week interval treatment. The treatments with a three-week sowing interval and L-first were on average 4.9% more productive in the 1.5 g m^{-2} density and 4.0% more productive within the 5 g m^{-2} density than their respective control, whereas there was no increase in productivity at 2.5 g m^{-2} (Fig. 2). Our experimental design was fully balanced except for the controls, i.e. all PFTs sown at same time, where we had three control replicates but one replicate was harvested at first harvesting date for the three-week sowing interval and the

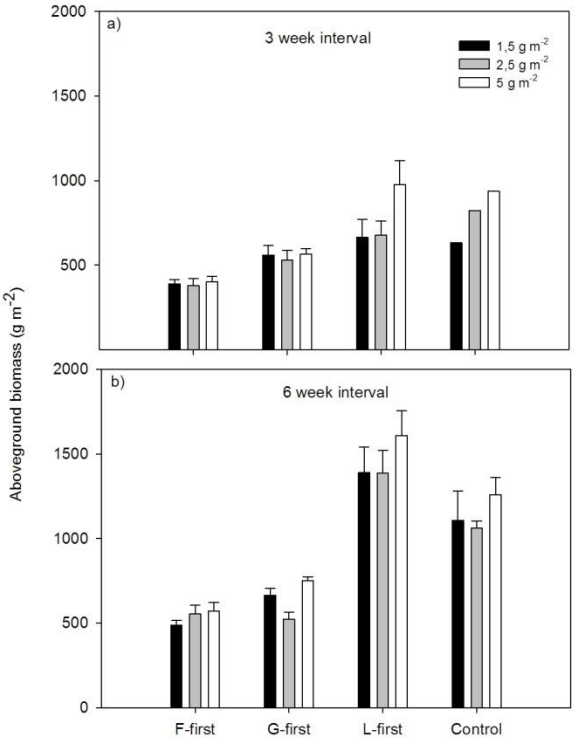

Figure 2. Sowing legumes first (L-first) produced the highest aboveground biomass, especially in the six-week interval treatment. Density had weaker effects on biomass than the priority effect treatment or sowing interval. Data show mean aboveground biomass (±1 SE) in the priority treatment separated into the three density levels. This is shown per sowing interval treatment with panel a) describing the three-week sowing interval and panel b) the six- week sowing interval treatment. For the priority effects treatment F, G and L-first = forbs-, grasses- and legumes-first. Replicates are n = 4 for main treatments and n = 2 or 1 for the controls in the six-week interval and three-week interval respectively.
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remaining two at the second harvesting date for the six-week sowing interval. This made sure that we allowed each plant community the same amount of time to develop after sowing. Taking this into account, the increase in productivity of the L-first group over that of the controls seemed not to be different between the three-week interval treatments (but no replication) but was significant in the six-week interval ($F_{(3, 38)} = 74.847$, $P<0.0001$).

Interactions were found between the factors priority effect and sowing interval ($F_{(3, 57)} = 7.466$, $P<0.0001$, see Table. 1). As a consequence, a Type I ANOVA was performed showing that irrespective of the sequence in which the other factors were fitted to the model, priority effect remained significant ($F_{(3, 57)} = 67.935$, $P<0.0001$).

Species & PFT Relative Abundances

The PFT sown first always dominated the functional composition of the plant community. Nevertheless, there was a clear difference between treatments with a three-week interval and a

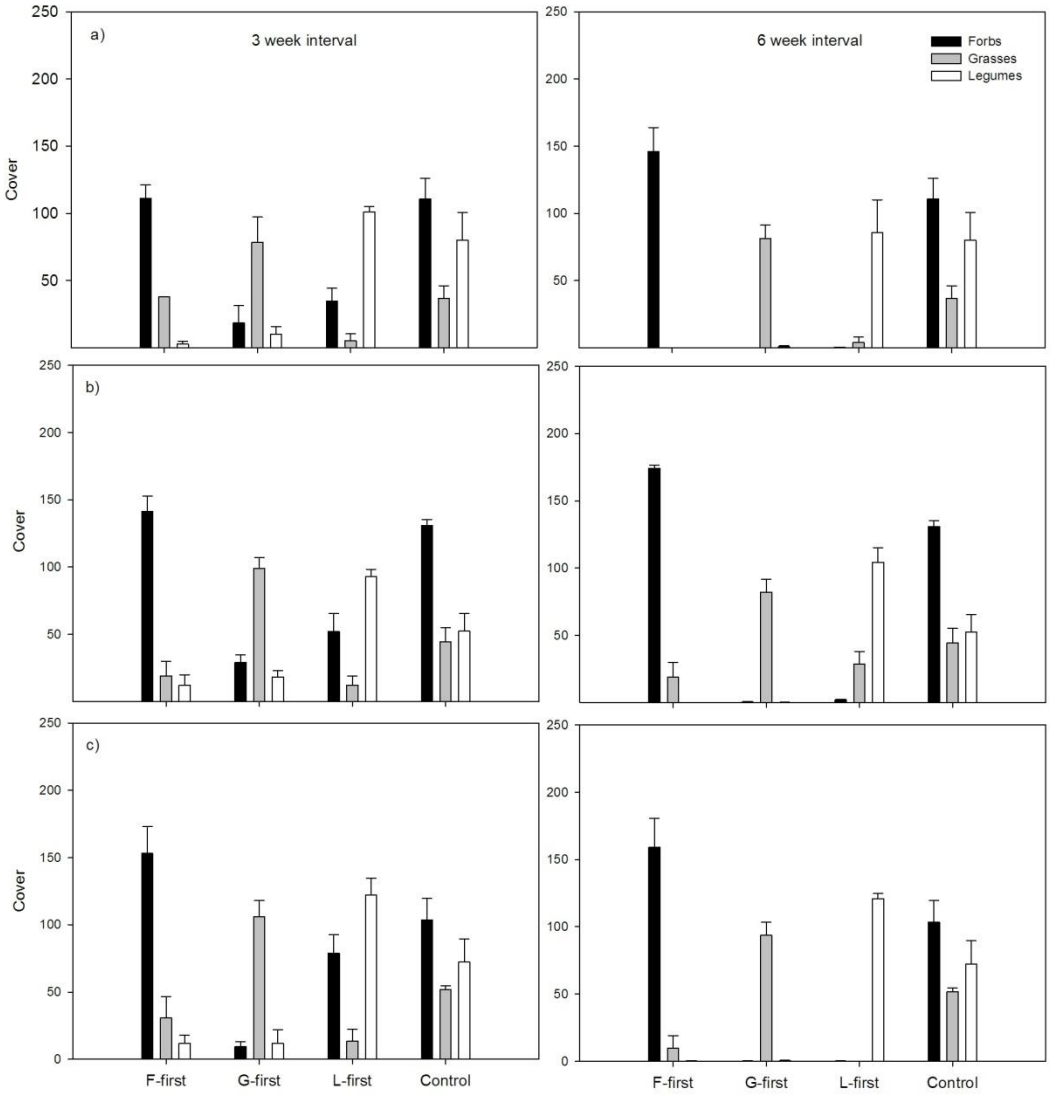

Figure 3. Effects of early sowing of one PFT (F, G and L-first = forbs-, grasses- and legumes-first) on the functional composition of communities in pots. Relative cover of PFTs (forbs, grasses, legumes) in pots were derived from individual species cover values summed and are depicted separately for each of the three densities: (a) 1.5 g m^{-2}, (b) 2.5 g m^{-2} and (c) 5 g m^{-2} for both sowing intervals (three and six weeks, in vertical columns) from vegetation assessments at peak biomass development. The data show mean values (±1 SE); n = 4 for all treatments (except for controls where n = 3).
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six-week interval. For treatments with a six-week sowing interval the relative abundance of the PFT sown first was nearly always > 90% except for one case (L-first treatment with a density of 2.5 g m^{-2} (78.6%)). The three-week interval treatment showed a more balanced relative abundance of PFTs. Priority effects on PFT abundance were consistent among the three density levels, favouring the PFT sown first with 73–84% relative abundance of forbs when forbs were sown first, 67–83% for grasses when grasses were sown first and 59–72% for legumes when legumes were sown first (Fig.3). Control treatments with simultaneous sowing showed forbs with relative abundances between 44–59%, grasses between 15–23% and legumes between 22–41%. Highest relative abundances in each PE-group were always in highest densities except for the L-first treatment where highest PFT relative abundance (72%) was at 1.5 g m^{-2}.

Within the L-first treatment subsequently-sown PFTs (grasses and forbs) were able to establish themselves better alongside the PFT sown first (legumes) compared to the other treatments (F- or

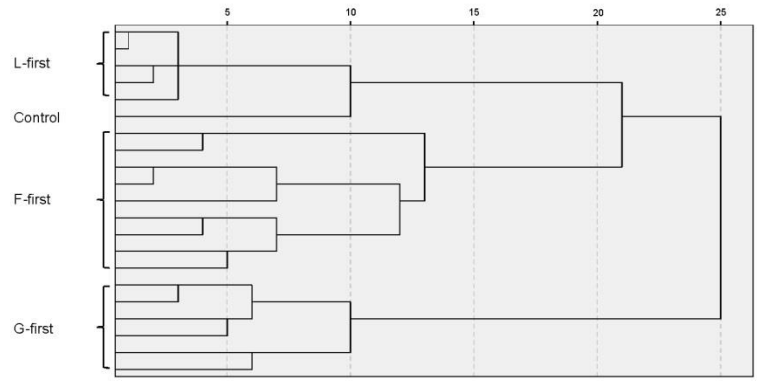

Figure 4. Effect of experimental treatments on the similarity of the resulting communities. Dendrogramm showing between group linkages for all replicates of the treatment groups: density (1.5, 2.5 and 5 g m^{-2}; sowing interval (3- and 6- week) and the priority effects treatment F, G and L-first = forbs-, grasses- and legumes-first as a result of a cluster analysis performed on data for relative individual species cover of every single pot in June 2011. As a measure of distance between groups Pearsons correlation coefficient was taken.
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G-first) where subsequently sown PFTs were suppressed (Fig. 3). This effect was stronger in the shorter sowing interval of three-weeks. Community development was clearly affected by the priority treatment and communities having the same starting PFT were more similar than those with different starting PFTs. A cluster analysis based on data on single species cover from vegetation assessments revealed three main groups in terms of species composition, and that these groups were mainly influenced by the starting PFTs. Most differences were found between communities with G-first and the rest, followed by a separation of the F-first group and a combined L-first and control group (Fig. 4).

Change in Soil Chemistry

Soil phosphate, nitrate and potassium were depleted by the end of the experiment when compared to values from the beginning of the experiment (T-test $P<0.05$; for details see Supporting Information, Table S4: Results of the T-test as a comparison of soil nutrient levels at the beginning and at the end of the experiment). In comparison to the nutrient content of soil samples collected at time zero, C/N ratios were higher at the end of the experiment than at the beginning ($t_{(82)} = 2.773$, $P<0.05$). However, no experimental treatments had any significant effects on the measured soil variables (for details see Supporting Information, Table S3: ANOVA performed on the effect of PE-treatment on soil variables).

Effect of Density on Aboveground Productivity

Density had a significant effect on aboveground productivity (Fig. 2; $F_{(2, 57)} = 5.567$, $P<0.05$) with a slightly higher productivity for the higher density levels. Nevertheless within the PE- and sowing interval treatments only a few treatments showed differences in aboveground biomass as a consequence of varying density.

For the L-first treatments and the three-week sowing interval, contrasts showed that the 5 g m^{-2} treatment had a significantly higher aboveground biomass compared to the lower sowing densities ($t_{(9)} = 2.143$, $P<0.05$). Within the G-first treatment similar biomass yields were found in all densities in treatments with a three-week interval but not in the six-week interval. Here the 2.5 g m^{-2} group was significantly less productive ($t_{(9)} = -3.975$, $P<0.005$) than the other two density levels.

The density treatment also influenced the number of individuals per pot, insofar as with increasing density the mean number of individuals increased across all treatments. Treatments with a sowing density of 1.5 g m^{-2} were having the lowest mean number of individuals ($t_{(66)} = 4.200$, $P<0.001$) and treatments with a sowing density of 5 g m^{-2} were having significantly higher numbers of individuals ($t_{(66)} = 2.841$, $P<0.005$) for both sowing interval treatments (Fig.5).

The influence of the priority effect-treatment was also visible in terms of numbers of individuals and showed the same trend for both sowing intervals. Treatments with a six-week interval had fewer individuals in each density level than in the three-week interval treatment ($t_{(67)} = 3.846$, $P<0.001$; Fig. 5).

Sowing Interval Effects

The effect of sowing interval on aboveground productivity between sowing of the first- and subsequent PFTs was significant (Fig. 2; $F_{(0.399)} = 59.313$, $P<0.0001$), with a sowing interval of six weeks showing increased productivity across all levels of the density treatment compared to the three-week interval. On average all treatments within the six-week interval group were more productive than the groups with a three-week sowing interval. The most pronounced difference in aboveground biomass was visible for the L-first treatment. In comparison (all sowing densities together) the L-first treatment with a six-week interval had 89% more biomass compared to the three-week interval group. The other PE groups for F- and G-first showed 38% and 16% increase in aboveground biomass respectively in comparison to the treatments with a three-week interval. Simultaneously sown controls were on average 62% more productive in the six-week interval group compared to the three-week interval control treatments.

The sowing interval also had strong effects on overall number of individuals per pot ($t_{(76)} = 3.588$, $P>0.005$; Fig. 5) and the overall plant species richness ($t_{(76)} = 4.376$, $P>0.001$) with lower individual and species numbers in the six week sowing interval.

Discussion

Our study found that priority effects of order of arrival at plant functional level had a substantial effect on aboveground productivity of sown communities, with L-first treatment being more

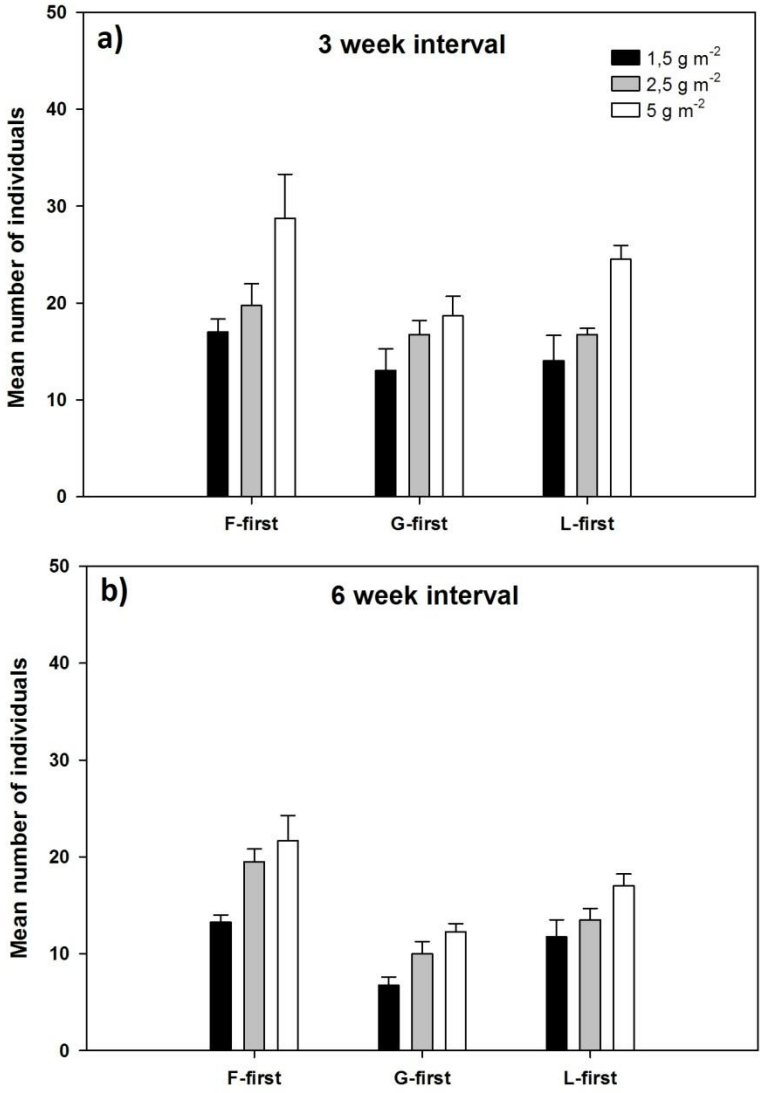

Figure 5. Effects of density and sowing interval on number of plant individuals per pot. For the priority effects treatment (PE) F, G and L-first = forbs-, grasses- and legumes-first. Values are mean number of plant individuals per pot with the PFT sown first on the x-axis and for all three densities for both groups sown with a three week interval (a) and a six week interval (b) between sowing of the first and remaining PFTs. (n = 4; ±1 SE).
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productive than controls sown at the same time or grass or forb species sown first (Figures. 2). These results (see Figure 4) confirm findings of Körner et al. (2008) and Ejrneas et al. (2006) in that the order of arrival of functionally different groups was critical to the development of their experimental communities resulting in clusters of different floristic distances to one another. In our study this is clearly reflected by the cluster analysis (Figure 4) on the basis of species' occurrence and relative abundances and the correlation to the functional composition of the resulting community. This analysis shows that the strong separation of communities was dependant on the starting PFTs which underlines the importance of priority effects in influencing the assembly process as found in a number of other studies [10,11,13]. Körner et al. [10] found that in terms of biomass production and final functional group composition after two growing seasons the L-first treatments and simultaneously sown controls were the most similar. Our experiment confirms this, even if our study ran for a shorter length of time and with a different species pool.

Our cover data confirm the aboveground biomass data in that in the L-first treatment, the functional groups were present in more balanced abundances than when grasses or forbs were sown first. Nevertheless, in the L-first treatment legumes contributed more to the overall community biomass than the other starting PFTs when they were sown first. L-first treatments were more productive than the other priority treatments irrespective of the sowing interval or sowing density, despite the fact that forbs were very dominant in relative cover and mean number of individuals. This suggests that legumes were better able to get a head-start and be productive before the later groups arrived even though species richness of the communities was rather modulated by the sowing interval (lower species richness when sowing interval was bigger) than by the identity of the species sown first, their traits allowed for better subsequent establishment of non-legume PFTs. In our experience legume species often do compete well and grow quickly in initial stages of experiments, as well as allowing for N facilitation with neighbours. Although legumes may not arrive earlier than other functional groups in naturally assembling communities, in ecological restoration we often wish to direct succession onto a desirable trajectory [34].

It seems that sowing legumes first led to asymmetric competition and fast growth of legumes [35] but at the same time more functional complementarity occurred between legumes and the other PFTs. A possible mechanism is the smaller rooting system (root mass fraction) of legumes if they are actively fixing atmospheric nitrogen, such that subsequent PFTs have more opportunities for both root space and nutrient foraging (also known as N sparing, [36]) and hence overall productivity is stimulated. Over a longer time span and under field conditions however, N facilitation (whereby the neighbours of N_2-fixers profit from legume-fixed N) may also cause higher productivity [37]. In this experiment treatments did not affect soil chemistry significantly even if C/N ratios changed from the beginning to the end of the experiment.

A likely explanation for the strong presence of forbs (at least considering cover and species numbers; Figures. 3&5) could be that forbs were overrepresented in species number right from the beginning (compared to the other PFTs there were 14 species sown within this functional group and only 7 for each legumes and grasses) to reflect the species and PFT composition common for restored grasslands in central Europe. But thus the F-first treatment had the lowest aboveground productivity, at least for our study no positive relationship between cover and productivity could be confirmed in this case (compare [27]).

We could not confirm the hypothesis that the longer the sowing interval the lower the aboveground productivity of the system will be. We hypothesised also that this would be because of stronger asymmetric competition between PFTs when early arrivers get a head-start and very little complementarity between PFTs can occur. What we found instead was that communities with a six-week sowing interval were more productive than those with a three-week interval (Figure. 2) despite the data showing higher mean species numbers (and also a higher species richness) in pots with a three-week sowing interval. A likely explanation would be that the starting PFT in the six-week interval group had three weeks longer to establish itself and grow than the three-week interval group. While the timespan for the two interval groups was the same after the second sowing occurred, meaning that for the two subsequently sown PFTs in every treatment the time allowed for growing was similar, the PFT sown first had 3 weeks more time to develop within the six-week interval. In general, later arriving PFTs contributed less towards community biomass as a consequence of the PE treatment and this makes sense since competitive advantage of the PFT sown first and thus asymmetric competition is part of the expected priority effect. Kardol et al. (2013) postulated that a priority-driven competitive advantage of early arriving species over later arriving species affected the probability of species coexistence and led to reduced species richness through competitive exclusion. This corresponds to our findings as we could also show a reduced number of individuals and lower plant species richness in the six week interval groups compared to the three week interval groups indicating the suppression of later arriving species by the PFTs sown first.

This could also be seen by looking at relative PFT contributions for the three- and the six-week interval (Fig. 3) where the six-week interval treatment was always particularly dominant without substantial contribution by the later sown functional groups species. We consider the starting PFT had a competitive advantage of arriving first and having better access to resources (especially light) before the competition with later arriving species occurred. As a result, niche space was likely filled more efficiently by the PFT sown first in the longer sowing interval treatments resulting in lower resource availability for later arriving plants as observed in other systems [38]. For a sowing interval of six weeks we observed an intensified dominance of the PFT sown first which was almost always above 90% in relative abundance at peak biomass whereas in the three-week interval, later sown PFTs were still able to compete and sustain a higher proportion within the communities.

Our hypothesis stated that because of the law of constant yield, sowing different seed densities will result in higher individual numbers at higher sowing densities, but overall aboveground productivity will remain the same across the different densities. Aboveground productivity did not differ across the density treatment but at the same time mean number of individuals per pot were significantly higher in treatments with higher sowing densities (Fig. 5). However, this difference did not strongly affect aboveground biomass and this suggests that soil nutrients were fully exploited by the community independent of how many individuals were present. As a consequence, higher sowing densities did not result in higher overall aboveground biomass, possibly because each individual was not able to be as productive as in lower density treatments, which is consistent with the law of constant yield [25]. It seems that the duration of our experiment was long enough for the law of constant yield to take effect.

Conclusion

The influence of assembly history on aboveground productivity was much stronger than sowing density or sowing interval (see Table 1 showing different effect sizes of factors). PE treatments led to the development of differently structured plant communities in terms of plant functional composition and dominance structure (Figs. 3&4). In natural succession plants often follow a sequence in which certain species establish and represent the community at a certain time point. This is often controlled by the local species pool and the availability of suitable environmental conditions for establishment. In our experiment both determinants were excluded (as often done in restoration practices) in the setup and thus the observed priority effect is of purely artificial nature. An important aspect of the priority effect was that the PFT sown first had significant effects on further functional composition with strong dominance of the early arriving PFT in the community. Although a larger sowing interval led to higher asymmetric competition we found evidence for complementarity between PFTs in the three-week interval treatment. In the latter, the cover of later arriving PFTs was larger than for other treatments when

legumes were sown first, suggesting that the optimal combination of functional groups would be sowing legumes first but making sure the sowing interval was not too long to enable the plants to achieve full maturity and thus have negative impacts on newcomers. Our results indicate that priority effects affect community development and function and that the severity of this impact seems to be much more driven by the question "Who comes first (and what is their function)?" than by questions like "when?" or "how many?". A possible application for our results can be found within the fields of restoration or agricultural practise when it comes to restoring ecosystem services or to increase productivity in low input high diversity systems [39,40]. To what extend we are able to set direction and to influence the development of plant communities via priority effects and their potential to create alternative stable states within plant communities is still to be addressed. So far to our knowledge no field experiments have tested these priority effects of functional group arrival time on community assembly and this would include a longer term and of course larger-scale assessment of priority effects on structure and function of communities. We are currently addressing this in a field experiment with the same kind of PFT-first treatments as in this pot experiment, where that we also find priority effects of sowing legumes early, even if one allows other species to invade aside from the sown species. Our study nevertheless confirms previous concepts of legumes as keystone species within N-limited grassland habitats, since the legumes seemed to have the ability to dominate at the same time as interacting with other groups in a complementary way [29,41]. Other studies have proposed asymmetric competition and plant-soil feedback effects as possible mechanisms behind priority effects (e.g. Grman and Suding 2010). Our study emphasises the need to also consider N facilitation effects of legumes as a driver of priority effects.

Supporting Information

Table S1 Plant species per functional group with respective seed mass per pot.
(DOCX)

Table S2 Results of soil analysis at the beginning of the experiment.
(DOCX)

Table S3 ANOVA performed on the effect of PE-treatment on soil variables.
(DOCX)

Table S4 Results of the T-test as a comparison of soil nutrient levels at the beginning and at the end of the experiment.
(DOCX)

Acknowledgments

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Author Contributions

Conceived and designed the experiments: VMT PvG NDJ CB UR CP. Performed the experiments: PvG CP VMT. Analyzed the data: PvG VMT. Contributed reagents/materials/analysis tools: VMT CB UR. Wrote the paper: VMT PvG.

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6.4 Manuscript 4

Time of arrival of different plant functional types during early assembly of experimental grassland communities affects above- and belowground productivity in a field experiment.

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Time of plant functional type arrival drives both belowground and aboveground grassland productivity during early assembly

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Abstract

Priority effects caused by species that arrive first during assembly can have long-lasting effects on both structure and functioning, but the role of the sequence of arrival of different plant functional types (PFTs) has not yet been investigated in under field conditions. In this grassland experiment, we tested the effects of arrival order of three different plant functional types (grasses, forbs, and legumes) as well as sowing either low or high diversity mixtures (9 or 21 species) on species composition and productivity (both above- and belowground) to reveal possible priority effects on assembly and ecosystem functioning during initial assembly of such grasslands. Both factors were tested on two sites representing different soil types (Cambisol and Anthrosol). The arrival order of PFTs influenced community composition in favor of species within the respective PFT sown first. Overall, plots in which legumes were sown first and control plots (sown at the same time) were more productive in aboveground biomass than grasses or forbs sown-first, and this was attributable to different functional compositions, especially legume dominance. Belowground we found opposite patterns of productivity: legume-first plots were least productive belowground versus grasses-first were most productive. Both above- and belowground productivity were modulated by soil type. No significant effects of sown species richness on aboveground productivity were found. The different biomass allocation patterns to above- and belowground tissues were clearly linked to the functional composition, in particular presence of dominant legumes. This in turn was controlled by priority effects induced by the experimental manipulation of the arrival time of different PFTs. The persisting dominance of species from the respective PFT sown first (despite the exclusion of aboveground competition by mowing before the second sowing) shows that there was interplay of two factors governing initial community assembly: belowground asymmetric competition leading to aboveground asymmetric competition.

Introduction

Priority effects during plant community assembly occur when species that establish first significantly affect further assembly and hence also following community trajectories. They can lead to lasting differences in species or functional composition (Ladd & Facelli 2008a; Grman & Suding 2010b; Fukami & Nakajima 2011a; Martin & Wilsey 2012b; Plueckers *et al.* 2013b) and hence can potentially drive local ecosystem properties and functioning (Fukami *et al.* 2006; Tan *et al.* 2012b; Kardol, Souza & Classen 2013a; Roscher *et al.* 2014). Priority effects can even have a stronger influence on community composition than abiotic conditions or resource availability (Chase 2003a). Thus, during the assembly of plant communities, a range of different community trajectories can be found which can lead to so-called "alternative stable states" (ASS) that are relatively stable at local alpha diversity scales (Temperton & Hobbs, R. J. 2004; Martin & Wilsey 2012b). While much is now known about the link between plant diversity and ecosystem functioning in experimental grasslands (Cardinale *et al.* 2007; Marquard *et al.* 2009b; Cardinale *et al.* 2013; Marquard *et al.* 2013) such experiments usually ignore effects of timing of arrival of different species or functional groups since species mixtures are sown or transplanted at the same time. If priority effects can play a key role in grasslands especially at alpha diversity scales , then the identity and effect of early arriving species at a site could be a key driver in affecting ecosystem functioning and ensuing diversity over time.

Ejrnaes, Bruun & Graae (2006) manipulated timing of arrival, fertility, soil and disturbance in grassland microcosms and found that species composition was mainly influenced by timing of arrival but also that "the probability for multiple equilibria appeared to increase with productivity and environmental stability". Also using grassland species, Kardol *et al.* (2012) found that timing of seed arrival affected plant community divergence and leaf chemistry but not community productivity or gas exchange and that the effects of timing of arrival were increased with soil fertility. Ejrnaes *et al.* (2006b) also found that assembly history influenced species composition of grassland- and generalist plant communities,

illustrating historical contingency (Belyea & Lancaster 1999), even if environmental factors influenced species richness and invasibility strongly.

Priority effects and plant functional types

The traits of species that establish first at a disturbed site can significantly influence further assembly with some species acting as inhibitors, facilitators or neutrally towards newcomers (Connell & Slatyer 1977b). Plants that establish first can gain a competitive advantage over species arriving later, altering resource availability at a site which in turn has an impact on the establishment and growth of species as well as on ongoing trajectories. Recently, controlled experiments have directly manipulated timing of arrival as a key component of possible priority effects, altering which plant functional types (hereafter called PFTs) arrive first (Ejrnaes *et al.* 2006b; Körner *et al.* 2007b; Kardol *et al.* 2013a; Gillhaussen *et al.* 2014b). Results of these studies indicate, that legumes sown prior to grasses and forbs create communities with higher aboveground (Körner *et al.* 2007b; Gillhaussen *et al.* 2014b) and lower belowground productivity (Körner *et al.* 2007). Körner *et al.* (2007) hypothesized that lower belowground productivity in treatments where legumes were sown first was due to smaller legume root systems (when deriving most N_2 from the atmosphere) and hence increased nutrient availability for non-legume neighbors arriving at a later time (the so-called N sparing effect, see Temperton *et al.* 2007). Clearly, seeding density and the sowing interval between sowing events has influence onthe early stages of community assembly but von Gillhaussen *et al.* (2014) found that sowing legumes before other PFTs created a stronger priority effect than sowing density or sowing interval did. However, none of these studies have shown how priority effects of PFT arrival affect community assembly and productivity (below- and aboveground) under field conditions.

Priority effects and the role of species interactions

Bullock *et al.* (2001a; 2007a), aiming to test whether positive diversity effects found in biodiversity experiments could be applied to the restoration of grasslands, sowed either low- or high diversity restoration seed mixtures (all species at the same time) on ex-arable land. They found that even under the same environmental conditions, initially sown, high species mixtures had a positive effect on both aboveground productivity and diversity over many years in 17 different restored calcareous grasslands (compared to sown low diversity mixtures). This can be seen as a priority effect on the whole system in terms of affecting the trajectory and functioning of the communities.

Complementarity between PFTs as well as assembly sequence is important for priority effects. Biodiversity experiments often find positive effects of plant species richness on ecosystem functioning particularly between specific combinations of functional groups (e.g. N_2-fixing legumes combined with grasses) (Hooper & Dukes 2004b; Kirwan *et al.* 2007a; Oelmann *et al.* 2007a; Marquard *et al.* 2009b). Positive interactions between legumes and other PFTs (as well as the extent of N sparing versus N transfer, *sensu* Temperton *et al.* 2007) are modified by the diversity and abundance of the interacting partners (Spehn *et al.* 2002; Temperton *et al.* 2007a) as well as by soil fertility (Märtin 2010). Complementarity (as well as competition) between functional groups occurs both above- and belowground (Marquard *et al.* 2009b; Ravenek *et al.* 2014a). So far, few studies have addressed the relevance of belowground processes during community assembly (but see Frank *et al.* (2010)).

Mommer *et al.* (2010) focused on vertical niche differentiation of root biomass of multi-species mixtures in comparison to monocultures in phytotrons. Already in the first year species in mixtures were on average more productive belowground than expected from monocultures, possibly preceding also aboveground overyielding. Ravenek *et al.* (2014) hypothesized that spatial niche differentiation in rooting patterns between plant species (and PFTs) may be a key driver of the observed biodiversity effect on belowground biomass, leading to more efficient overall belowground resource-use with

increasing diversity. They reported a positive effect of diversity on standing root biomass (albeit with a time lag of four years) but no evidence for spatial niche differentiation. In contrast, strong evidence for positive effects of grasses and negative effects of legumes on standing root biomass were observed (Ravenek *et al.* 2014). Whether biodiversity can promote the productivity of agriculturally managed grasslands and how it interacts with priority effects during initial assembly is, however, largely unexplored (Isselstein, Jeangros & Pavlu 2005b).

To investigate the effects of order of arrival of different PFTs as well as sown diversity effects, we set up a field experiment with these two factors tested on two different soil types. Additionally, we were interested in finding out whether former results from greenhouse experiments (Körner *et al.* 2007b; Gillhaussen *et al.* 2014b) could be confirmed under field conditions. Since Bullock *et al.* (2007) and Bezemer & van der Putten (2007a) found strong priority effects of sowing different degrees of diversity on productivity and established species richness, we hypothesize that sowing low vs high diversity as well as changing the order of arrival of PFTs (as in the controlled experiment of Körner *et al.* 2007) may create large priority effects. Any such effects, if desirable, could be used to improve the restoration outcome in species-rich grasslands, thus ensuring that their overall productivity remains high over a long period of time and creating a win- win situation among conservationists and farmers (by creating a highly diverse system with low input and high productivity).

The experiment has a multi-factorial design with order of arrival of different plant functional types and species richness tested on two different soils for their effects on above- and belowground productivity as well as on composition of the emerging communities. The following hypotheses were tested:

1) Sowing legumes first creates a priority effect by increasing aboveground community biomass yield and negatively affecting overall belowground root productivity.
2) Initial species richness creates a priority effect, which increases aboveground biomass yield in the high diversity mixtures.
3) Soil type modulates overall productivity but priority effects will have a stronger effect on productivity irrespective of soil properties.

Materials and Methods

Experimental site

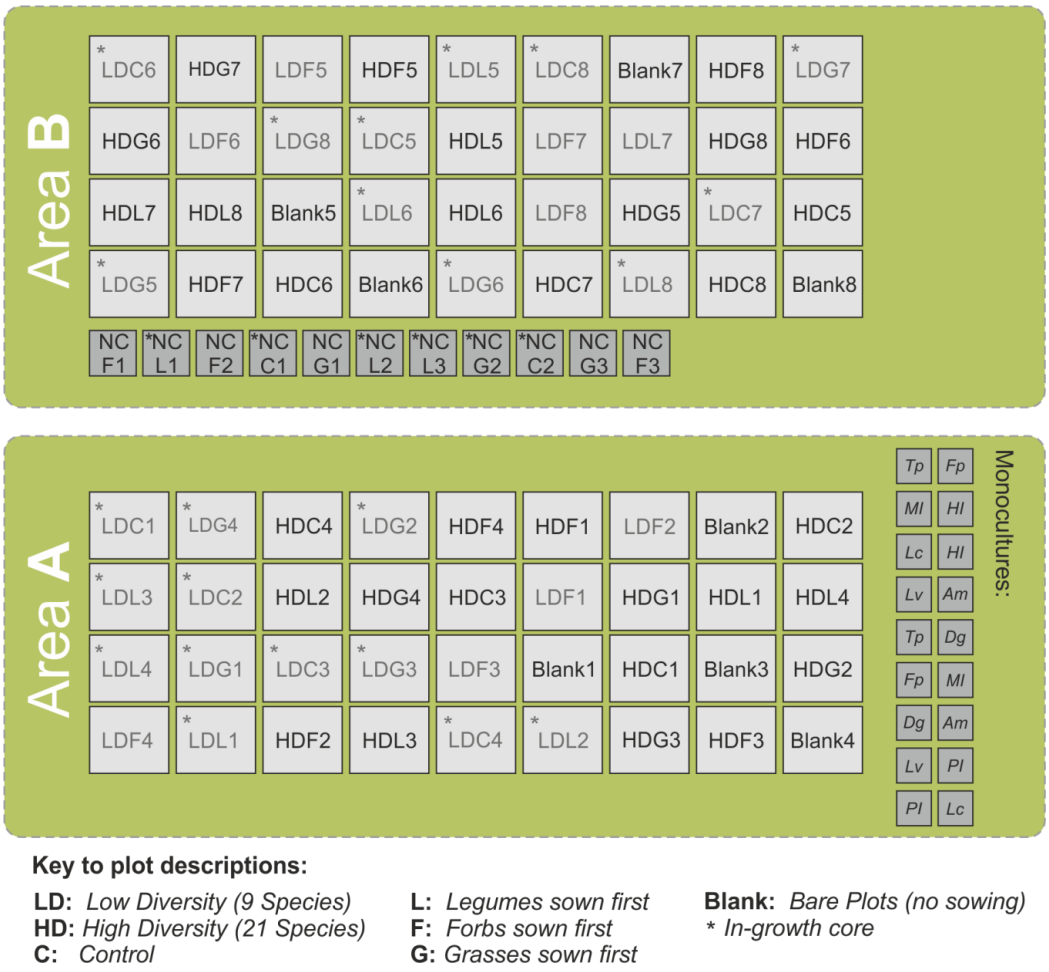
The Priority Effect Experiment is located on an ex-arable field southeast of Jülich (in North Rhine-Westphalia, Germany) near the village of Daubenrath (altitude 94 m NN; 50°53'51.53" N, 6°25'21.09" O). Mean annual air temperature at the nearest meteorological station (approx. 1 km distance from the field site) is 9.9°C (1961-2012) and mean annual precipitation of 699 mm. The site was cultivated as an arable field until 2006 (mainly for the cultivation of vegetables and root crops) and was then used as extensive grassland from 2006 until the establishment of the experiment in 2012. Prior to the establishment of the experiment the field was ploughed and raked multiple times during the winter 2011/2012 to counteract germination of weeds from the soil seed-bank and to create bare ground.

Before the start of the experiment a soil survey was conducted in December 2011 where 72 soil samples were taken from the Ap horizon using an Auger corer in every plot of the later established main plots in a 4 x 4 m grid. The soil samples were stored at -18°C and later analyzed for mineral nitrogen (N_{min}: nitrate, ammonia), C_{tot}, N_{tot}, Ca_{tot}, Fe_{tot}, K_{tot}, Mg_{tot}, Mo_{tot} and pH of the soil solution extract ($CaCl_2$).

Additionally an assessment on the basis of four soil profiles (two on Area A and B respectively) was performed, where soil type, grain size and soil skeletal content were assessed. As a result of the soil survey, the experiment was set up on two areas (Area A and B see Fig.1) reflecting the soil types Stagnic Cambisol on area A (depth of > 140cm with nearly no soil skeleton in the first 30 cm) and a slightly elevated (app. 1.8m), piled up Anthrosol (depth of >150, with a soil skeletal content of 10-25% in the first 30cm). The soil survey followed the official German soil mapping guidelines (Sponagel 2005).

Experimental Design

a)



Key to plot descriptions:

LD: *Low Diversity (9 Species)*
HD: *High Diversity (21 Species)*
C: *Control*

L: *Legumes sown first*
F: *Forbs sown first*
G: *Grasses sown first*

Blank: *Bare Plots (no sowing)*
** In-growth core*

b)

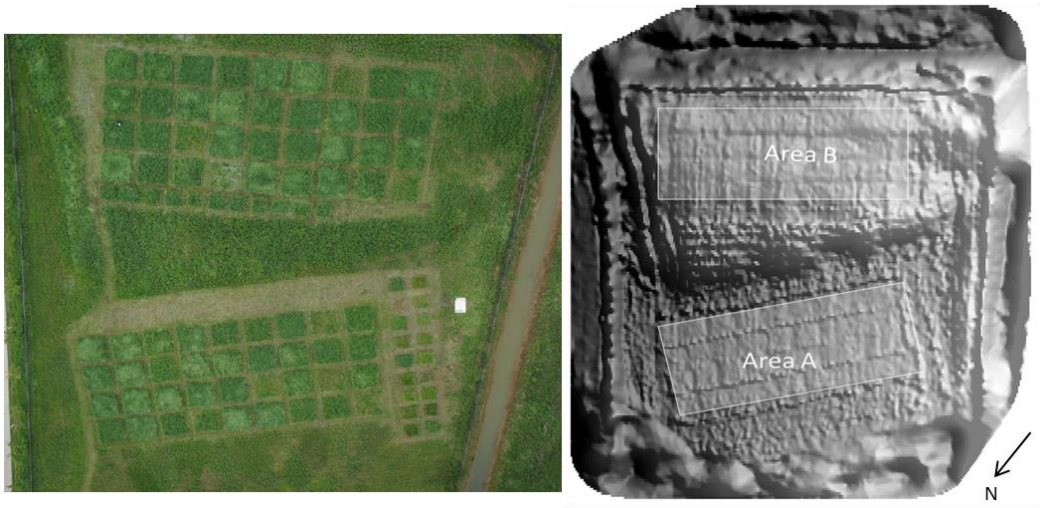


Figure 1: a) A schematic representation showing the distribution of plots and treatments on area A and B and b) an arial image (lower left) and a digital elevation model (right image) depicting differences in elevation between area A and B. Plot descriptions

represent either sown diversity (HD, LD) treatments or PFT order of arrival treatment (F: forbs, G: grasses, L: legumes and C: simultaneously sown controls) and the respective number of replicate. N= 4 per Area. The grey plots in a) next to area A shows 18 monoculture plots, with two plots (2x2m) per species for all species contained in the low diversity seed mixture. The grey rectangle next to area B shows 11 "No-cutting" plots where no mowing between the 1st and 2nd sowing occurred, providing a test of whether priority effects occur when aboveground competition is not reduced between the two sowing events (data from monocultures and "No-cutting" plots not shown here).

The field experiment is multi-factorial with the factors sown diversity and PFT order of arrival as the two main factors. Treatment levels were high- (HD) and low diversity (LD) for sown diversity and grasses-first (G-first), legumes-first (L-first) and forbs-first (F-first) or control (sown at the same time) for PFT order of arrival. The experiment covers two different soil types, separated by a slight elevation, giving two different areas, Area A and B (see Figure 1), each with exactly the same treatment factors and replicates (n= 4 per Area, n= 8 for the whole experiment, resulting in a total number of 72 plots). The two different seed mixtures ("high diversity" and "low diversity") were sown in the experiment to assess the effects of species richness on ecosystem functions and diversity outcomes in the assembling communities (see also Bullock *et al.* 2007). All plant species in the low diversity mixture were a subset of the species contained in the high diversity mixture (see Table 1). All plots were mown twice per growing season (according to agricultural practice in managed mesic grasslands). In 2012 mowing was executed on July 30th and October 4th since the experiment was establishing itself in this first year and peak biomass was later than usual (normally in May). A non-clonal grass species, *Festuca rubra spp. commutatis*, was sown in the areas between the plots as lawn paths.

Table 1: Plant species chosen for the Priority Effect Experiment with the respective PFT (column 1) assigned for each species (column 2). Species were selected from a species pool of the typical central European grassland types. Species pools for high and low diversity (HD and LD) mixtures were fixed (not random). Species contained in the low diversity mixture are shown in column 3.

PFT	Species	Present in LD plots	Present in HD plots
Forb	*Achillea millefolium*	1	1
Forb	*Crepis biennis*	0	1
Forb	*Galium verum*	0	1
Forb	*Glechoma hederacea*	0	1
Forb	*Leontodon hispidus*	0	1
Forb	*Leucanthemum vulgare*	1	1
Forb	*Plantago lanceolata*	1	1
Grass	*Arrhenatherum elatius*	0	1
Grass	*Bromus erectus*	0	1
Grass	*Dactylis glomerata*	1	1
Grass	*Festuca pratensis*	1	1
Grass	*Helictotrichon pratense*	0	1
Grass	*Holcus lanatus*	1	1
Grass	*Poa pratensis*	0	1
Legume	*Lotus corniculatus*	1	1
Legume	*Trifolium hybridum*	0	1
Legume	*Trifolium pratense*	1	1
Legume	*Medicago sativa*	1	1
Legume	*Onobrychis vicifolia*	0	1
Legume	*Lathyrus pratensis*	0	1

The PFT order of arrival treatment was created by sowing the species of one PFT first on 19.04.2012 (or all at the same time for the control) and the other species of the two remaining PFTs at the same time on 31.05.2012, resulting in the four treatment levels: F-first, G-first, L-first and control (all PFTs sown at the same time). The length of the interval between sowing events was based on previous greenhouse

studies (see von Gillhaussen *et al.* 2014) where 6-week intervals produced larger priority effects than a 3-week interval. Before the 2nd sowing was done all plots were mown with a lawnmower at a cutting height of 30 mm, to reduce initial aboveground competition and to allow subsequently sown species to better germinate and establish. This was performed in order to increase complementarity between PFTs. None of the plots was weeded thus allowing colonization and assembly processes after the one sowing event. In addition to the sown plots, four blank plots were established where no seeds were sown, as a free succession control. The main experiment thus consists of 72 plots of 4x4 m. In addition, 18 smaller plots of 2 x 2 m were also established with monocultures of the low diversity treatment species (replicated twice).

Species selection

Seeds were obtained from a local regional source within North Rhine-Westphalia (Rieger-Hofmann GmbH, Blaufelden-Raboldshausen, Germany), mixed manually to the correct seed mixture. Each seed mixture, except for the simultaneously sown control groups, was further separated into a first- and second sowing mixture, for the PFT order of arrival treatment. The seed mixtures were mixed with sand to ensure a proper handling and a more even distribution of seeds on the plots at the time of sowing. The target sowing density was 5 g m^{-2} divided equally among the species of each mixture. The number of PFTs within each of the seed mixtures was always equal (high diversity: forbs: 7, grasses: 7, legumes: 7 or low diversity: forbs: 3, grasses: 3, legumes: 3) and the number of seeds taken for each species was adjusted according to their thousand seed weight. Seeds were sown by hand into the previously prepared (raked) plots at a sowing distance of approx. 30 cm above ground level to avoid seed distribution by wind. Afterwards each plot was flattened with a 50 kg roller to ensure proper adherence of seeds to soil particles and to avoid herbivory.

Species selection was supposed to reflect species that were relatively dominant and common in grasslands of the surrounding area. The target plant community (Arrhenatherion) is a semi-natural

species rich mesophilic grassland, consisting of typical central European grassland species (Ellenberg 1988). In total, a fixed set of 21 common species (7 forbs, 7 grasses and 7 legumes) was selected for the high diversity communities. A randomly chosen, fixed subset of 3 forbs, 3 grasses and 3 legumes was selected to represent low diversity communities (see Table 1). Species were selected taking their performance in previous experiments (e.g. (Pywell *et al.* 2003; Gillhaussen *et al.* 2014b) and pre-experiments into account.

. Species were classified into three different plant functional types (PFTs) and this was intentionally held broad, to create general functional envelopes in which plant species' in all likelihood would differ significantly in their functional and morphological traits (based on Roscher *et al.* 2004 for the Jena Experiment, except that forbs were not split into small and tall forbs). We defined three different PFTs: (non-legume) forbs, grasses and legumes. Forbs included any non-legume, non-grass species; grasses included members of the Poaceae family which are morphologically most different from the other groups (common prevailing traits of this group are a perennial life cycle and a caespitose growth). Legumes are forbs of the Fabaceae family which vary from species of other PFTs by their ability to fix atmospheric nitrogen and use it as a nitrogen source.

Sampling and Data Collection

The core area within every plot (3.5 x 3.5 m) was not used for any other sampling except biomass harvesting and species- specific cover estimates. To identify treatment effects on plant community composition plant cover per species was estimated prior to the harvest of aboveground biomass. These estimates were performed using a modified cover estimation method following Braun Blanquet (Londo 1976).

Total aboveground biomass production (dry matter yield, g/m^2) was measured at two harvest events in 2012 (31.07 – 03.08 and 04. – 05.10.2012). Two 0.1 m^2 rectangles (20 × 50 cm), randomly positioned

each time, were harvested in each plot at each harvest. All aboveground plant material within the rectangle was cut approx. 2 cm above the soil surface and samples were dried at 70°C (until constant weight) before weighing. After biomass-sampling all plots were mown with a lawnmower at 80mm, cut plant material was removed from the plots. The outer area of each plot (approx. 60cm wide) was designated as a "measurement zone" where destructive measurements (e.g. soil samples) were possible without interfering with overall community establishment and assembly.

Assessment of fine root productivity, standing root biomass and root decay

Root growth and turnover were estimated with the aid of the ingrowth-core (IGC) method (Steen 1984; Hansson, Steen & Andren 1992; Steingrobe, Schmid & Claassen 2000) in the low diversity G-first L-first and control treatments. Prior to the sowing (on 05.04.2012) mesh bags (Polyamide fiber, length 45 cm, mesh size 1 mm, diameter 4 cm) were fitted into pre-drilled holes (diameter 5 cm) at an angle of 45° to soil surface, covering a soil depth of approx. 30 cm. Four IGCs were installed in each plot with a distance of at least 1 m between each bag. Prior to their activation the IGCs were protected by inserting PVC-tubes (diameter 4 cm) into the mesh bags. All non-active IGCs were covered with plastic caps. The soil material used to fill the IGCs was taken from an area next to the experimental plots with corresponding soil properties. Before soil material was collected the surface of this area was covered with an opaque plastic film for 10 weeks to suppress any plant growth and to obtain soil material free from living roots. Subsequently, soil material (0-30 cm depth) was extracted, air-dried (for 3 days inside a greenhouse at 20°C) and sieved (2 mm). The first set of IGCs was filled on 23.05.2012. The mesh bag of each ICG was stepwise filled by repeatedly pulling out the PVC tube for a few centimeters, inserting a small quantity of soil material pushing it into the ICG and compacting it with a wooden stick. In order to achieve a soil density inside the mesh bags, similar to that of the surrounding plot, the dry bulk density was determined previously and an according soil quantity was filled into the ICGs. At the time of activation weed plants in an area of 50 x 50 cm around the IGCs were carefully removed to ensure that only roots

of the sown species were included into the assessment. A detailed description of the Ingrowth core method is provided by Steingrobe *et al.* (2000; 2001).

After activation the IGCs remained inside the soil for two weeks where fine root ingrowth into the mesh bags was allowed. Only one IGC at a time was active inside the plots reflecting the fine root productivity of a certain 2 week period (from here on called generation). Whenever one generation of IGCs was sampled, a reference soil core was taken from the plot for the examination of overall standing root biomass. Root decay inside the IGC during the 2 weeks exposure period was considered negligible, thus total root length inside the IGCs was considered to represent total root productivity for the given 2-weeks period and soil volume of the cores (Steingrobe *et al.* 2001). After one generation had been sampled the next generation was activated to allow a continuous monitoring of fine root productivity for a total of 8 weeks over the time span from 23.05 to 18.07.2012. Sampling dates were 06.06. (1^{st} generation), 20.06. (2^{nd} generation), 04.07.2012 (3^{rd} generation) and 18.07.2012 (4^{th} generation). After two weeks of active exposure, mesh bags were pulled out and the roots were washed out carefully over a 500 µm sieve. Then root length was determined by a line intersection method according to Newman and Tennant (Newman 1966; Tennant & Tennant 1975). Measured root length was related to the volume of the IGCs allowing the calculation of the average root length density (RLD, cm root per cm^3 soil). Root decay was calculated as follows: $(RLD_{core}(time2) + RLD_{ref}(time1)) – RLD_{ref}(time2)$, where RLD_{ref} is the root length density of the reference cores (standing root biomass) and RLD_{core} is the root length density inside the IGCs.

Statistical analysis

The field experiment is multi-factorial in design with the fixed factors **Sown diversity** and **PFT order of arrival** as the two main factors and **Area** as a fixed factor. Sown diversity had two levels: high (HD: 21 species) and low diversity (LD: 9 species). The factor **PFT order of arrival** had four levels: grasses-first (G-first), legumes-first (L-first) and forbs-first (F-first) or control, sown at the same time. The two Areas with

two different soil types had exactly the same treatment factors and replicates (n= 4 per area, n= 8 for the whole experiment, giving a total number of 36 plots per Area and overall 72 plots for the whole experiment, see Figure 1C). Whenever the factor Area had a significant effect, separate analysis for area A and area B were performed.

Biomass data (aboveground and belowground) were analyzed using ANOVA testing for effects of the factors sown diversity and PFT order of arrival and the Area treatment (Area A and B) as well as any interaction effects between these factors. The experimental design was balanced and orthogonal for the three factors. Data was analyzed using Type III ANOVA. Post-hoc tests (Tukey HSD) were used to identify significant differences between treatment levels and where appropriate outcomes of Tukey tests are reported.

Normal distribution of the residuals and homogeneity of variance were checked with pp-plots and Levene's tests respectively. Any data that did not fulfill the assumption of homogeneity of variance and normal distribution of the residuals were transformed (log 10) before analysis. Effect sizes for each factor as the proportion of explained variance were calculated as partial η^2. Details of the results for separate analysis (e.g. t-tests or ANOVAs for specific harvesting dates) which are not listed in Table 2 are stated in the text. Analyses were run using PASW Statistics 22 (IBM Corporation, New York, USA).

Analysis of species compositional data was performed by a PCA on the basis of Bray Curtis dissimilarities on behalf of species specific cover estimates on all plots (irrespective of Area or Sown diversity) for the two dates of cover estimates July and September. These analysis were run using R studio (Version 0.98.1062 – © 2009-2013 RStudio, Inc.) using the extension software package "Vegan".

Results

Effects of PFT order of arrival on aboveground biomass production

The treatment PFT order of arrival had a significant effect on the total aboveground biomass in 2012 (data from both harvests pooled: July and October; Fig. 2; $F_{(3, 94)}$= 22.064, P<0.0001). The Area in which the experimental communities were growing in (A or B) also had a significant influence on aboveground biomass ($F_{(1, 94)}$= 10,424, P= 0.002; with higher biomass on Area A. In contrast, Effects of sown diversity on aboveground biomass were not significant, even if higher diversity seed mixtures did tend to produce slightly more productive community biomass (Fig.2). Interactions of the treatments PFT order of arrival and Area as well as between the treatments PFT order of arrival, sown diversity and Area on aboveground biomass production were found, therefore the data was analyzed separately for the two areas showing that PFT order of arrival remains significant (see Table 2).

Table 2: ANOVA results for testing the effects of Sown diversity, PFT order of arrival and Area on different response variables. Note that this summary table is compiled of several different analyses, some that relate to the full set of experimental plots (N=8) or a subset of plots on Areas A and B (N=4). Results for the subset of plots with in-growth cores installed for measuring belowground productivity and standing root biomass are reported under the respective entries. Each (thick) line represents a separate ANOVA test. Interactions effects are denoted with a X. P values < 0.05 are considered statistically significant and shown in bold.

Response variable	Factor	df	F-value	p-value	partial η2
Total Aboveground biomass (g/m^2)	Sown diversity	1	0,133	0,716	0,001
	PFT order of arrival	3	22,064	**0,000**	0,413
	Area	1	10,424	**0,002**	0,100
	Sown diversity x Order of arrival	3	0,409	0,747	0,013
	Sown diversity x Area	1	0,012	0,913	0,000
	PFT order of arrival x Area	3	3,267	**0,025**	0,094
	Sown diversity x Order x Area	3	2,992	**0,035**	0,087
Aboveground biomass (g/m2) on Area **A**	Order of arrival	3	15,283	**0,000**	0,534
	Sown diversity	1	0,034	0,854	0,001
	Sown diversity x Order of arrival	3	1,041	0,385	0,072
Aboveground biomass (g/m2) on Area **B**	Order of arrival	3	11,551	**0,000**	0,391
	Sown diversity	1	0,118	0,733	0,002
	Sown diversity x Order of arrival	3	2,393	0,078	0,117
Fine root productivity (cm/cm3)	PFT order of arrival	2	1,948	0,149	0,042
	Area	1	7,288	**0,008**	0,076
	PFT order of arrival x Area	2	2,691	0,073	0,058
Fine root productivity (cm/cm3) on Area **A**	PFT order of arrival	2	2,492	0,097	0,125
Fine root productivity (cm/cm3) on Area **B**	PFT order of arrival	2	1,949	0,157	0,098
Standing root biomass (cm/cm3)	PFT order of arrival	2	6,195	**0,003**	0,121
	Area	1	0,565	0,454	0,006
	PFT order of arrival x Area	2	3,372	**0,039**	0,070
Standing root biomass (cm/cm3) on Area **A**	PFT order of arrival	2	10,117	**0,000**	0,310
Standing root biomass (cm/cm3) on Area **B**	PFT order of arrival	2	0,504	0,607	0,022

When treating the different harvesting events separately, the effect of PFT order of arrival on aboveground productivity was significant at the first harvest in July ($F_{(3,\ 58)}$= 9.652, P<0.0001) and remained significant for the second harvest in October ($F_{(3,\ 51)}$= 5.783, P= 0.002).

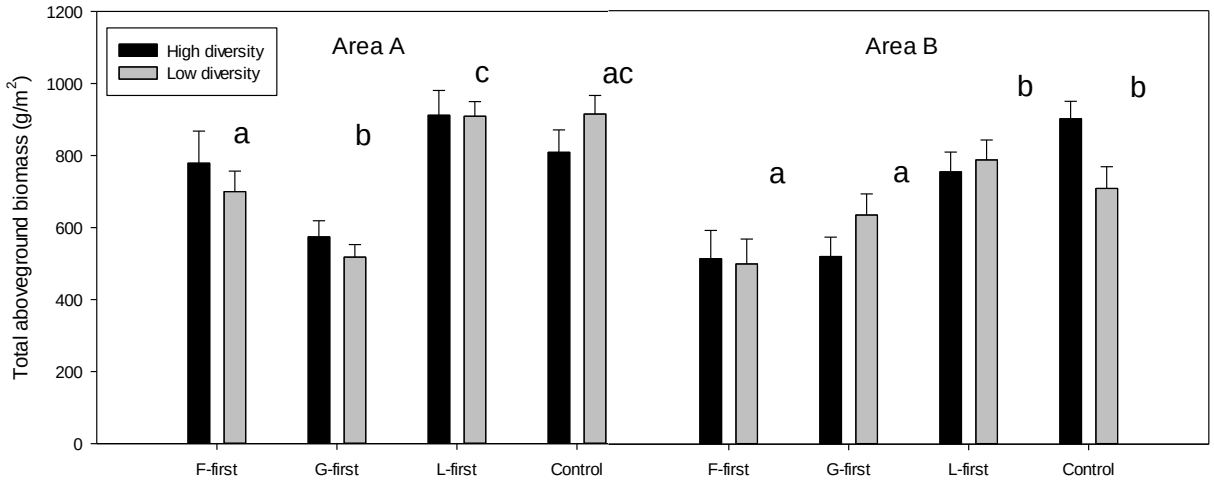


Figure *2:* Total aboveground productivity over the growing season 2012 (biomass in July and *October* 2012 summed). Bars show high diversity plots (black) and low diversity plots (grey) separated into PFT order of arrival treatments with forbs, grasses-, legumes sown first (F-, G-, L-first respectively) and simultaneously sown controls. Panels show results from area A and B, high diversity and low diversity plots combined for Post-Hoc tests. Data are means (+/- 1SE). N=4 per *Area*.

Aboveground and belowground productivity during initial eight weeks of assembly in a subset of plots where in-growth cores were installed

We compared belowground productivity from the subset of plots sampled with aboveground productivity of the same subset of plots to make the comparison more exact. PFT order of arrival had a significant effect on aboveground productivity for the subset of plots where IGCs were installed (LD plots) during the first eight weeks of the experiment (see Fig.3; $F_{(3, 24)}$ =3.947, P=0.020). When Areas A and B were analyzed separately there was a significant effect of PFT order of arrival on aboveground productivity in area A ($F_{(3, 13)}$ =5.753, P= 0.01), but not in Area B ($F_{(3, 11)}$ =1.620, P= 0.241). Aboveground productivity was highest in Control and L-first plots, which were significantly higher than G- or F-first plots ($F_{(3, 28)}$ =3.724, P<0.0001).

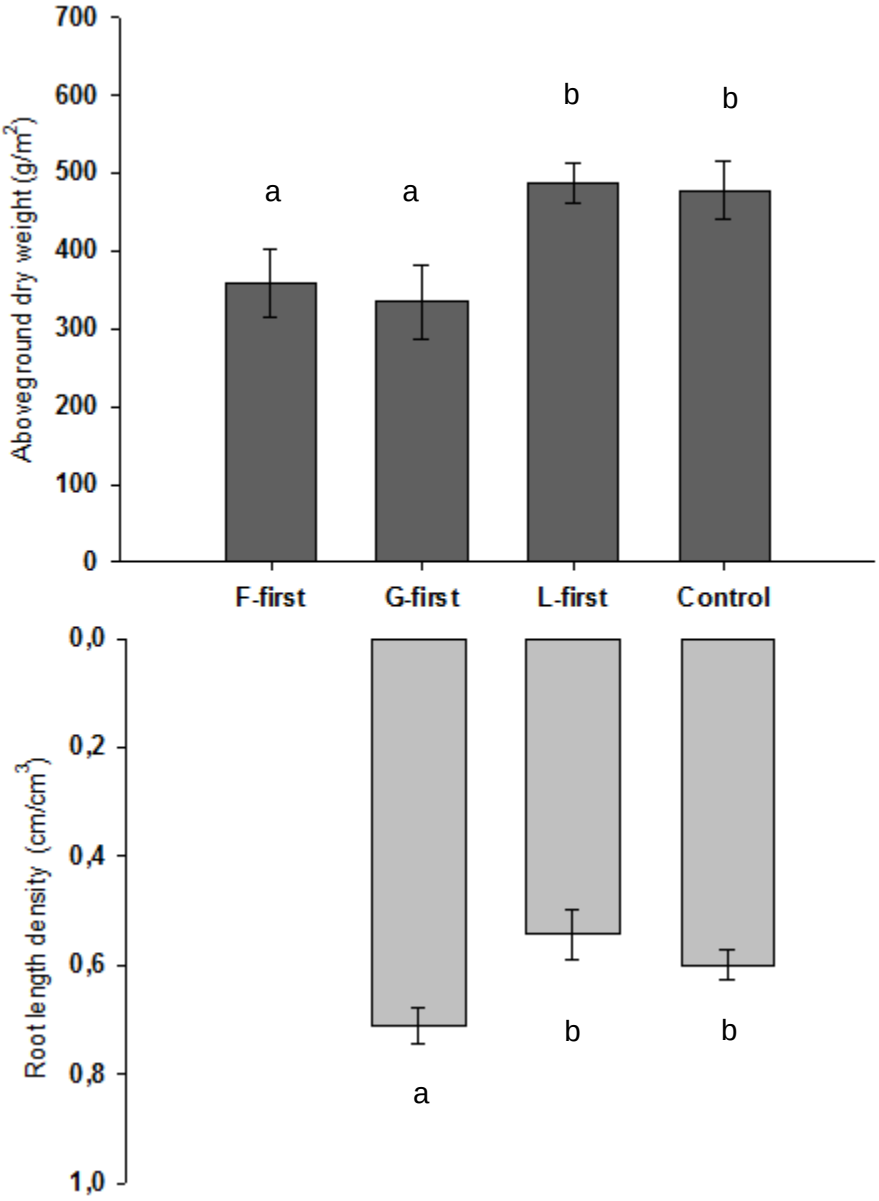


Figure 3: Plots with legumes sown first produced highest aboveground- (P<0.0001) but lowest belowground biomass (standing root biomass; P= 0.008) in reaction to the PFT order of arrival treatments. Data show mean aboveground biomass and root length densities (± 1 SE) for the first eight weeks of the experiment. All levels of the PFT order of arrival treatment (F-, G- and L-first = forbs-, grasses- and legumes-first) are shown for a subset of plots (all low diversity mixtures) where ICGs had been installed (except F-first where no ICGs had been installed). Replicates are N= 8.

Belowground, PFT order of arrival had a significant effect on standing root biomass of reference cores ($F_{(2,95)}$= 7.004, P= 0.001; sampled additionally to IGCs in bulk soil adjacent to the IGCs). Mean root length

densities for L-first and Control plots were significantly lower than in G-first plots (($t_{(61)}$= 2.753, P= 0.008; see Fig. 3). Mean root length densities derived from standing root biomass from reference cores (across all sampling dates) were highest for plots with grasses sown first (G-first) with 0.712 +/-0.217 cm/cm^3 followed by the controls with 0,619 +/-0.180 cm/cm^3 and L-first plots with 0.544 +/-0.186 cm/cm^3 (Fig. 3). Figure 4 shows the belowground root dynamics of plots where IGCs were installed over the four different sampling dates (generation 1-4). Figure 4a shows that for G- and L-first initial standing root biomass diverged after the first sampling date (Generation 1) with G-first becoming more productive compared to L-first plots where belowground biomass remained at a constant level. A significant difference between these two PFT order of arrival treatment levels was found from the 3rd generation on ($t_{(14)}$= -3.021, P=0.009) and at this time also between the G-first and Control treatment ($t_{(14)}$= -3.134, P= 0.007). At the time of the fourth IGC generation, L-first standing root biomass was significantly lower than in G-first ($t_{(14)}$= -2.157, P=0.049).

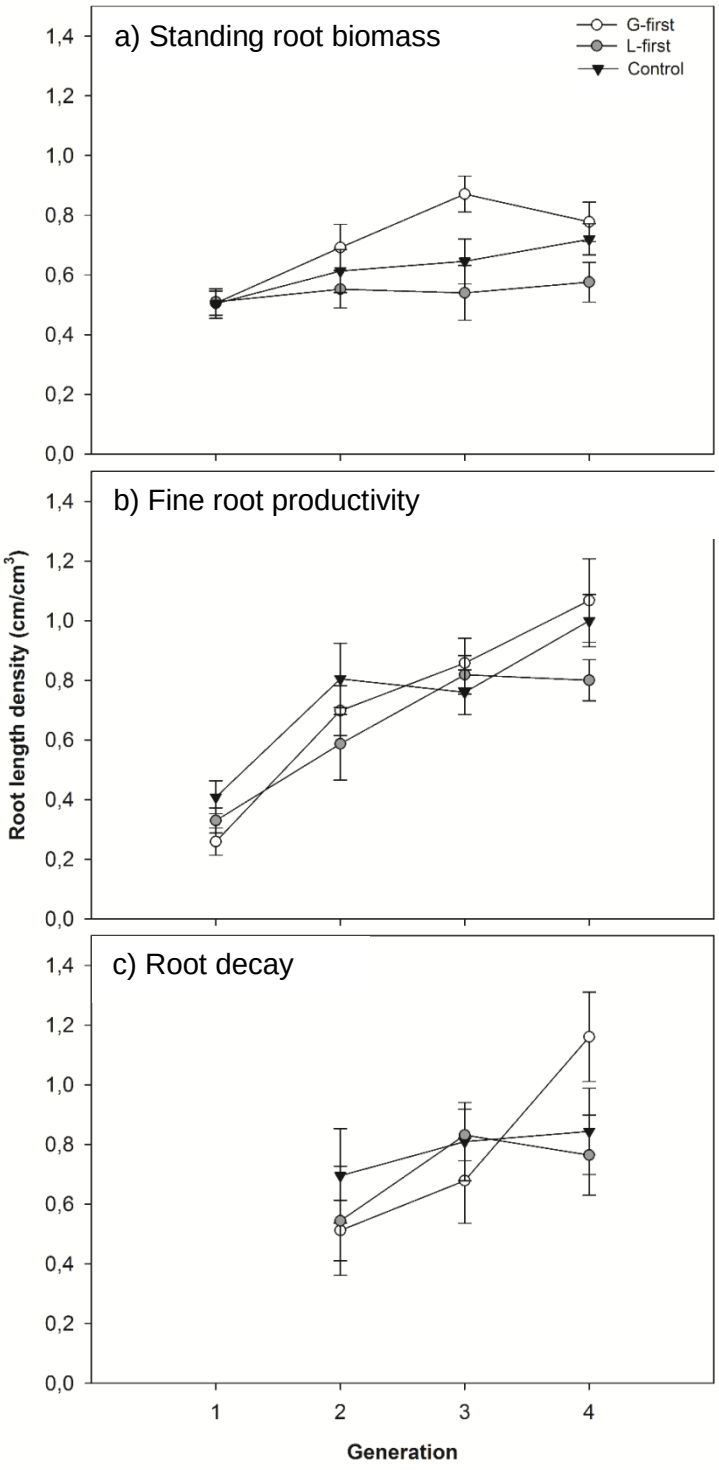


Figure 4: Mean root length densities (cm/cm³) of a) standing root biomass (taken from bulk soil samples), b) fine root productivity (taken from ingrowth cores) and c) root decay across all replicates of the treatments G-first, L-first and Control (grasses- or legumes sown first and simultaneously sown controls; N = 8, +/- SE) for each of the four generations, each reflecting a period of two weeks of ICG exposure and possible root ingrowth.

PFT order of arrival did not show effects on total fine root productivity ($F_{(2\ 94)}$= 1.948, P=0.149) significantly (Figure 4b and Fig. 5, Table 2). Nevertheless, looking at data across all 4 generations, L-first was the least productive belowground with an overall mean of 0.634 +/-0.075 cm/cm^3 followed by the simultaneously-sown controls with 0.743 +/-0.130 cm/cm^3 and grasses sown first (G-first) as the most productive treatment level with 0.759 +/-0.222 cm/cm^3. Despite finding no significant effect of PFT order of arrival on fine root productivity (see Tab.2), the pattern of productivity over time (Fig. 4b) reveals differences. L-first plots decreased in fine root productivity during the last two sampling dates (shortly before and while flowering from 04.07.2012 (3rd generation) to 18.07.2012 (4th generation)) compared to the G-first plots, leading to an overall lower productivity. The simultaneously sown controls experienced a decrease in productivity between the 2nd and 3rd generation (sampling dates 20.06. and 04.07.2012) but were able to compensate towards the end of the sampling period. Only plots with grasses sown first managed to keep up a constant productivity until the final sampling (18.07.2012).

In this context, accumulated root decay (Fig. 4C) was also lowest for L-first plots with 2,140 +/-0,356 cm/cm3, followed by the controls with 2,349 +/-0,415 cm/cm3 and G-first with 2,351 +/-0,675 cm/cm^3 (not significant).

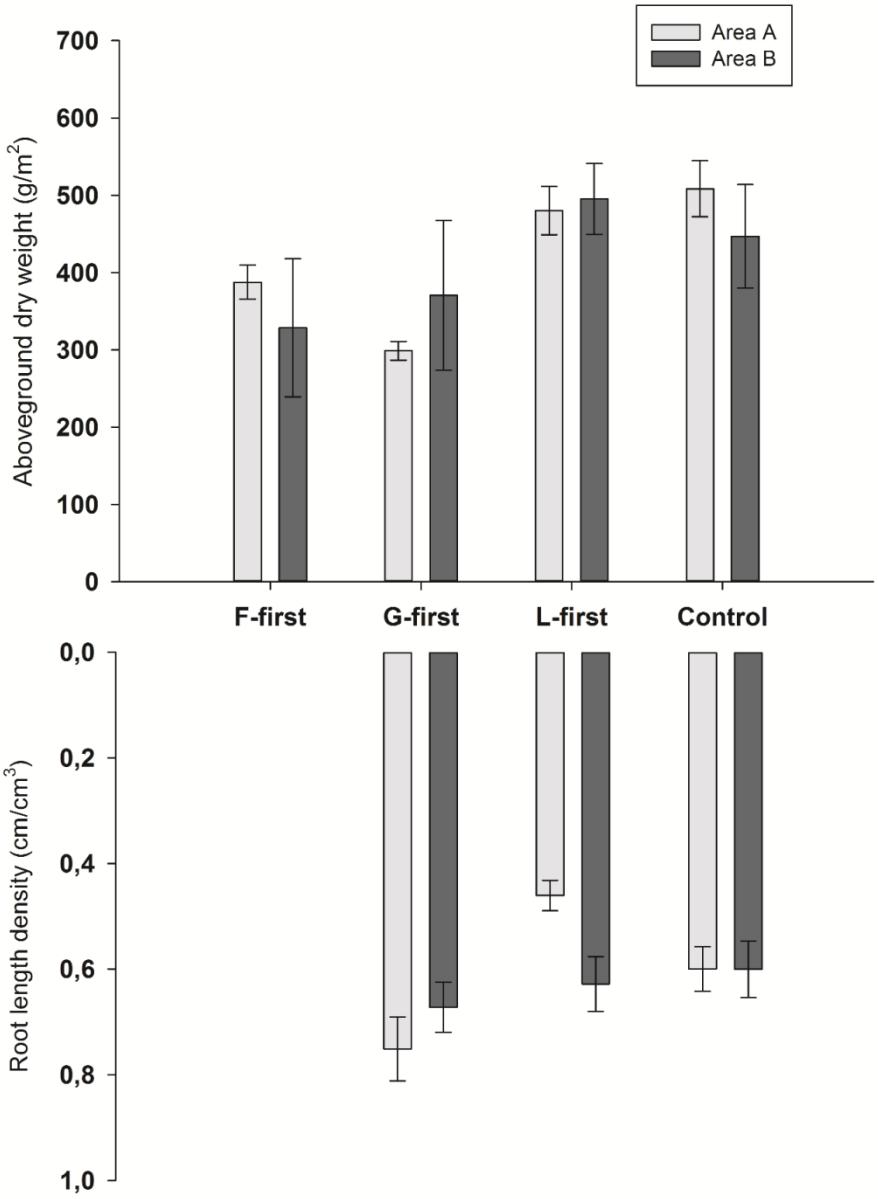
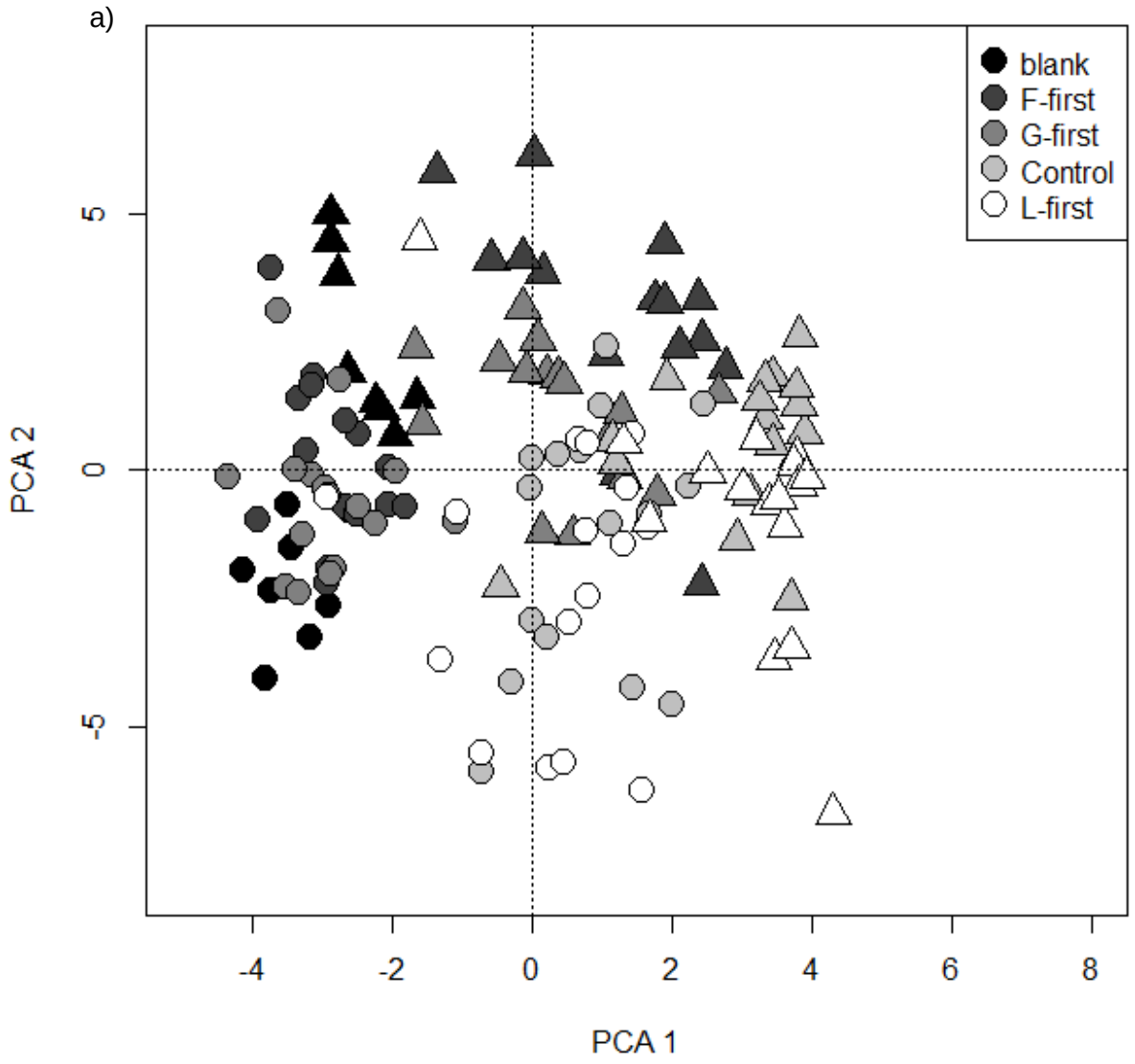


Figure 5: Different soil conditions affecting productivity above- and belowground (fine root productivity only, as a mean across sampling generations, +/- SE) during initial assembly (first eight weeks of the experiment). Data shows all levels of the PFT order of arrival treatment (F-, G- and L-first = forbs-, grasses- and legumes-first) for a subset of plots (all low diversity mixtures) where ICGs had been installed (except F-first where no ICGs were installed), divided among the two blocks A and B. Replicates are N= 4 per block.

Effects of treatments on aboveground functional composition

The PFT order of arrival treatment resulted in different functional compositions for the experimental plots: Fig. 6a shows (Bray Curtis-) dissimilarities in community composition based on species specific

cover estimates of plots in July (circles) and September (triangles) and one can see that 1) the community functional composition changed strongly between the two time points and 2) L-first and control plots cluster on the one hand and G-first and F-first plots cluster in a separate multivariate space reflecting the same pattern previously seen on aboveground biomass and standing root biomass (compare also Fig 2 and 3).



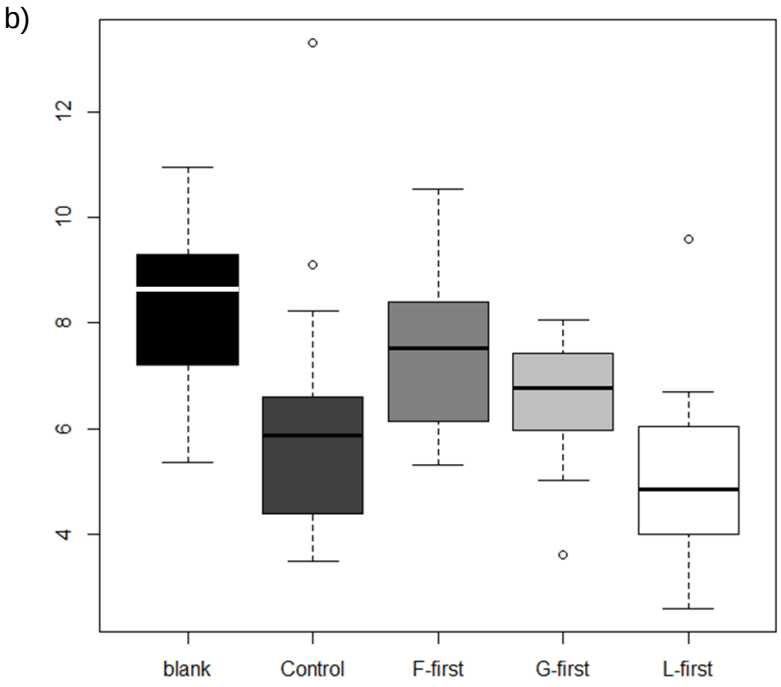


Figure 6: a) Ordination showing the effects of PFT order of arrival treatment (F, G and L-first = forbs-, grasses- and legumes-first) on the functional composition of communities in plots (irrespective of sown diversity and soil type). Axes resulted from PCA of Bray Curtis similarities of experimental plots derived from individual species cover estimates for the two time points in July (circles) and September (triangles). b) Box-plots showing Euclidean distances (functional dissimilarities) between the first (July) and the second (September) cover estimate for the different PFT order of arrival treatments.

At peak biomass in July species of the PFT sown first were already substantially contributing to community biomass, with sown legume species in L-first plots holding a share of approx. 43% of the total community, in F-first plots, sown forbs were approx. 15% of the total community and in G-first plots, sown grasses contributed approx. 12% to the total community. Controls consisted of approx. 38% sown legume species, 10% sown forb species and 4.5% sown grasses (Area and Sown diversity treatments pooled), confirming the patterns in Fig. 6a) whereby the control plots had similar compositions to the L-first plots.

Fig. 6b) shows Euclidian distances of functional compositions between the two time points July and September for each level of the PFT order of arrival treatment, showing that the compositional stability during these three months is higher for L-first and Control plots than for the others. Control plots and plots in which legumes were sown first were more legume-dominated whereas plots with grasses or forbs sown first were initially dominated rather by annual forbs and grasses (weeds; however, this effect was substantially reduced after the first mowing).

A linear regression of legume abundance and aboveground biomass showed that legume abundance was positively correlated with aboveground biomass production throughout all plots of the experiment ($R= 0.558$, $P<0.001$ for July; $R= 0.460$, $P<0.001$ for October).

During the initial establishment weed pressure from the soil seed-bank was relatively high and led to high non-target species abundances. Non-target species abundances before the first cut, in July was highest in G-first plots (approx. 83%) followed by F-first plots (approx. 79%), L-first plots (approx. 54%) and Control plots (approx. 47%) but decreased strongly as a response to cutting after the first harvest and the subsequent mowing in the end of July. At the time of the second cover estimate in September 2012, weed species abundances had already decreased strongly (G-first: 40.6%, F-first: approx. 22.8%, L-first: approx. 21%, Control: approx. 12%).

Effect of sown diversity on productivity and species composition

The treatment sown diversity (HD vs LD) did not have any significant effect on aboveground productivity, neither on total productivity ($F_{(1, 94)}= 0.133$, $P= 0.716$, see Table 2) over the whole season nor at either single harvest in July ($F_{(1, 48)}= 0.888$, $P= 0.351$) or in September ($F(1, 51)= 2.416$, $P=0.126$). Although no significant effect of the sown diversity was found, there was a trend visible towards higher productivity in high diversity plots (see Fig.2). Total productivity in 2012 for low and high diversity mixtures was 18286.7 +/- 198.4 g/m^2 and 20625.5 +/- 221.9 g/m^2 respectively.

On average the sowing of high diversity seed mixtures significantly affected species richness within sown communities, with a higher target species richness (target-SR: 8, total-SR, (including weeds): 24) as well as overall species richness compared to the low diversity plots (target-SR: 5, total-SR: 20; $t_{(61)}= 3.995$, $p < 0.001$). The number of non-target species remained unaffected by sowing species richness (LD-plots: 15, HD-plots: 16 non-target species, in October).

Effects of soil type

As an ANOVA factor the treatment Area had a significant effect on total aboveground productivity of plots (Table 2) with plots on Area A having a slightly higher overall biomass compared to plots on Area B (Fig. 2. These significant differences between Area A and Bwere related to soil type and structure. T-tests on further soil parameters measured revealed that soils in Areas A and B (Fig. 1) differed significantly in N_{tot}, pH (CaCl2), Ca_{tot} and K_{tot}, with higher N concentrations and pH-values in Area B.

As a consequence, Area-specific differences were also found for belowground. The effect of order of arrival on standing root biomass varied betwwen Area A and B. Additionally the factor Area had a significant effect on belowground productivity of fine roots (see Table 2).

Discussion

Overall, effects of PFT order of arrival were much stronger than effects of sown species richness, and order of arrival effects on productivity were modulated by soil type (see Table 2). Tested for the first time under field conditions the early sowing of legume species 5 weeks before sowing the rest of the community led to an increased aboveground productivity at community level (compared to grasses or forbs sown first; but not compared to the control sown at the same time). During early assembly legumes dominated in the control plots as well as in L-first plots and these plots were functionally similar compared to functionally different species compositions in G-first and F-forb plots It seems therefore that the higher aboveground biomass in the L-first and control treatments was mainly driven by the dominance of legumes in these treatments during early assembly in the first growing season.

Effects on aboveground productivity

Legume abundance was positively correlated with aboveground productivity across all main plots of the experiment, alsosuggesting that legumes were driving aboveground productivity during early assembly in this experiment. This has been found in other similar successional or grassland field experiments albeit with species sown at the same time (Bezemer & van der Putten 2007a; Marquard *et al.* 2009b; Roscher *et al.* 2011). Von Gillhaussen *et al.* (2014) suggested that in the L-first treatment, species sown after the legumes may have a better chance of establishing as a result of the smaller rooting systems of legumes (*sensu* Körner *et al.* 2007) as well as via nitrogen (N) facilitation. Our field results, although not as strongly significant as those found by Körner *et al.* (2007) under controlled conditions, show that grasses and legumes are performing very differently as drivers of grassland assembly (regarding the allocation of above- and belowground biomass). And to what extent they drove assembly did depend on when they arrived in relation to other functional groups.

European grassland legumes are known to have high growth rates and thus can establish quickly aboveground although over time, grasses tend to outcompete legumes as their root foraging advantage takes hold (Neugschwandtner *et al.* 2013). One possible priority effect of sowing legumes first could be that this engenders more N facilitation either via direct N transfer from legumes to neighbors or via so-called N-sparing whereby the non-fixing neighbors take up more of the soil N as the legumes rely more on atmospherically fixed N. N facilitation could hence lead to increases in productivity aboveground. If N facilitation were playing a role we might expect to see some positive effects of legume early arrival in terms of more balanced functional composition of plots in L-first treatments and/or higher N concentrations in the soil or in non-legume neighbors, which was not the case (and stable isotope data on a few select species sampled in Oct 2012 revealed little evidence for N facilitation during initial assembly in 2012; data not shown). This is in strong contrast to a pot priority effect experiment with a very similar approach where we found that L-first pots had a more balanced functional composition than other treatments (von Gillhaussen *et al.* 2014).

Effects belowground: standing root biomass and fine root productivity

Our experimental treatments caused effects on standing root biomass differently to fine root productivity (Table 2, Fig.4a and b): standing root biomass was significantly affected by PFT order of arrival whereas fine root productivity was not. In contrast, fine roots productivity was significantly different between Area A and B, but not between PFT order of arrival treatments (Table 2; Fig.4a and b).

Fine root productivity in plots where legumes were sown first was lower than where grasses were sown first, illustrating differences in belowground biomass allocation patterns and growth rates of legumes and grasses previously already documented by others in biodiversity experiments where species were all sown at the same time (Gastine, Scherer-Lorenzen & Leadley 2003; Ravenek *et al.* 2014a)

Belowground standing root biomass of communities revealed that legume dominated plots (L-first and C) had lower overall root biomass compared to other treatment levels already from the first generation of sampled IGCs onwards (Fig. 4a), suggesting that the dominant legumes (namely *Trifolium pratensis, Lotus corniculatus* and *Medicago sativa*) drove overall community root biomass throughout the investigated period of eight weeks. In addition, grass-dominated plots had not only higher fine root productivity but also higher fine root decay, which indicates faster root turnover and thus increased belowground competitiveness due to a faster preemption of possible resources (Fransen, Kroon & Berendse 2001; Frank *et al.* 2010).

In line with the reduction in fine root productivity, root decay in L-first plots was lowest between the two last sampling dates, which is no surprise as with the IGC method root decay is a function of productivity.

Carlsson *et al.* (2009b) could show that legumes, when growing simultaneously with neighbors of other functional types, increase their reliance on N_2-fixation per unit plant biomass, hence possibly allowing for more N-sparing to occur as a form of N facilitation benefit for non-legumes. Although grasses tend to be the better competitor for belowground resources this does take time (Neugeschwendtner et al. 2013), legumes produced a much higher share of the total biomass during early assembly and legume abundance was positively correlated with aboveground productivity. Our findings suggest that during early assembly (after a disturbance) legumes can dominate over grasses (if not sown after other PFTs), whereas as succession proceeds the grasses can slowly pre-empt soil and light resources (Kirmer, Baasch & Tischew 2012; Neugschwandtner & Kaul 2014).

Plant-plant interactions above- and belowground

Competition aboveground is particularly asymmetrical when some species arrive earlier than others as some species get a head start casting shade on any seedlings of later arriving species and thereby negatively affecting growth conditions for smaller plants (Weiner 1990). Whether or not belowground

competition is asymmetrical in natural ecosystems is still unclear (Frank *et al.* 2010). Besides aboveground asymmetric competition we experimentally induced asymmetric competition belowground as a consequence of our time of arrival treatment and particularly due to mowing at the time of the second sowing. The already established aboveground biomass from plants of the PFTs sown first was cut at that time while leaving belowground plant parts intact which probably led to a strong reduction of aboveground asymmetric competition. Nevertheless, species of the PFTs sown first continued to stay dominant in the further course of community development and dominance even became stronger with time and cutting events during 2012 (see Fig. 5). A possible explanation may be that as a result of cutting, the relative abundance of non-target species was reduced and target species could generally establish themselves better. A more likely explanation could be that a once established root system is a competitive advantage (Brouwer 1983; Kroon, Mommer & Nishiwaki 2003) particularly when most of the competitors (from other PFTs sown later) still have to establish a root system. Kroon et al. (2003) found that when aboveground plant parts had been cut, species which were sown first regenerated more quickly from disturbance, than seedlings from PFTs sown later. This could lead again to aboveground asymmetric competition, which in turn suggests that the interplay of above- and belowground competition are the driving mechanisms behind observed dominance patterns within priority effects (Grman & Suding 2010b). Our study indicates that belowground effects may be key drivers during the creation of observed priority effects (since the aboveground difference between L-first and control was not significant but belowground it was (Figure 5 in Area A).

Effects of sown diversity on productivity

Although sown diversity did not significantly positively influenced aboveground productivity as found in many biodiversity experiments, (e.g. van der Putten and Bezember 2007 and Bullock *et al* 2008) in most cases in our study high diversity plots were slightly more productive compared to low diversity plots (Fig. 2). We could show, however, that sowing of a species mixture with higher species richness resulted in

more species-rich communities, as also found by Bullock *et al.* (2007). Our lack of a significant species richness effect may be attributable to the realized species diversity not being very different between treatments, with 8 vs 5 target species in high vs low diversity treatments and 24 vs 20 total species richness respectively (probably due to non-target "weedy" species emerging from the soil seed-bank rather than arriving by wind dispersal).

Kirmer, Baasch and Tischew (2012) found that sowing high diversity seed mixtures versus low diversity grass cultivars on surface-mined land in Germany enabled faster successional development and effects of the sowing event were still detectable 6 years later, despite migration of species from the high diversity to the low diversity treatments. It remains to be seen for how long the priority effects found in our experiment will last compared to such restoration-related experiments.

Effects of soil type and structure

Plots in Area A and B differed significantly (Table 2) in total aboveground productivity and were relatively consistent in their response to the PFT order of arrival treatment (Fig. 2), with a slightly higher productivity on Area A. This higher productivity may be due to soil properties on Area B which are less optimal due to the higher soil skeletal content and higher exposure to wind (resulting from the slight elevation compared to Area A) even if soil N content was found higher in Area B.

Likely, the disparity in soil characteristics (especially in skeletal content) also led to differences in belowground productivity of fine roots and standing root biomass for the two Areas A and B (Scott Russell 1977).

The lower elevation of plots on Area A and the proximity to a nearby forest may have favored the arrival and establishment of non-target species together with the older age of the soil on Area A may have led to a larger seed bank (Li *et al.* 2012).

Conclusions

Priority effects caused by the early arrival of different PFTs have not yet been studied under experimental field conditions and we could show that belowground dynamics of root productivity can be key drivers of any such priority effects. The legume-first priority effects we found, particularly belowground were predominantly independent of soil type or sown diversity, even if soil type did modulate the priority effect. Additionally for the first time we show effects of arrival order of PFTs on belowground productivity in the field and find that different biomass allocation patterns of PFTs may translate into different outcomes regarding community structuring. We could also show that legumes dominated those plots that had higher aboveground productivity and lower belowground productivity, namely those where legumes were sown first or at the same time as the other PFTs. An important possible implication of these finding is that legumes are able to dominate during early assembly of such grasslands when either sown first or at the same time as other PFTs, but not when sown after other PFTs. Results may be relevant to application in the context of grassland restoration or agricultural management of marginal lands. If such priority effects are able to create alternative stable states, they could possibly be used to "steer" ecosystem functioning onto desired trajectories of relative functional composition. Thereby we could gain influence on the provision of different ecosystem functions and services i.e. a certain level of productivity at the same time as being relatively species-rich or others such as erosion control, improved water retention and carbon sequestration.

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8. Declarations

(Eidesstattliche) Versicherung und Erklärung

(§5 Nr.4 PromO)

Hiermit erkläre ich, dass keine Tatsachen vorliegen, die mich nach den gesetzlichen Bestimmungen über die Führung akademischer Grade zur Führung eines Doktorgrades unwürdig erscheinen lassen.

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